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FLUVIAL SEDIMENT ORGANIC MATTER DEGRADATION IDENTIFIED WITH ELEMENTAL AND ISOTOPIC FATE DURING LABORATORY INCUBATION

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FLUVIAL SEDIMENT ORGANIC MATTER DEGRADATION IDENTIFIED WITH ELEMENTAL AND ISOTOPIC FATE DURING LABORATORY INCUBATION

THESIS

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science in Civil Engineering
in the College of Engineering at the University of Kentucky

By

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Lexington, Kentucky

Director: Dr. James Fox, Professor of Civil Engineering

Lexington, Kentucky

2020

ABSTRACT OF THESIS

FLUVIAL SEDIMENT ORGANIC MATTER DEGRADATION IDENTIFIED WITH ELEMENTAL AND ISOTOPIC FATE DURING LABORATORY INCUBATION

Fluvial sediment is well recognized as a critical factor in both carbon and nutrient budgets within stream systems. However, we find very few studies of reactivity and isotope enrichment for stream water from agricultural and urban streams and the class of substrate known as fluvial sediment organic matter. This study investigated the hypothesis that fluvial sediment is subject to degradation even though many previous studies have considered this class of substrate generally inert. Therefore we qualify that elemental and isotopic signatures of fluvial sediment organic matter should be considered potentially non-conservative when used in tracer studies. Methods applied to this research project included field measurements, laboratory incubation experiments, and numerical modelling. Sediment and water samples were analyzed to determine the elemental concentration of carbon and nitrogen, as well as isotopic ratios of oxygen, nitrogen, and carbon in order to (1) elucidate the fate of carbon and nutrients during elemental decomposition and spiraling as well as isotope fractionation, (2) investigate the role of biotic processes in transforming nitrogen and carbon, (3) and combine the data results with a kinetics model that incorporates knowledge of biogeochemical processes in streams. Results of this study suggest a moderately active system dominated by dissolved- and sediment-organic carbon oxidation, CO_2 evasion, nitrogen mineralization, and nitrification. Best estimates of isotope enrichment factors ranged from -3 to +1‰ for dissolved- and sediment-organic matter oxidation, -1 to +1‰ for nitrogen mineralization, and 0.05 to 0.2‰ for nitrification. While biochemical processes are occurring, results suggest lack of isotopic enrichment during carbon oxidation, nitrogen mineralization and nitrification.

KEYWORDS: Fluvial sediment, oxidation, organic carbon, nitrogen, laboratory incubations

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06-16-20

FLUVIAL SEDIMENT ORGANIC MATTER DEGRADATION IDENTIFIED WITH ELEMENTAL AND ISOTOPIC FATE DURING LABORATORY INCUBATION

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Chapter 1 - Introduction

1.1 Research Needs and Motivation

The research literature review, methods, results and discussion in this paper fulfill two major research needs in the environmental water resources research fields. These two research needs focus on reactivity and isotope enrichment for purposes of fluvial sediment fate and the conservativeness, or lack thereof, of tracers in fingerprinting. These research needs are summarized in the next sub-sections.

1.1.1 Research Need #1

Reactivity and isotope enrichment for stream water and fluvial sediment organic matter for C and N freshwater cycles/budgets:

We find very few studies of reactivity and isotope enrichment for stream water from agricultural and urban streams and the class of substrate in stream water known as fluvial sediment organic matter. However, these are highly uncertain organic matter pools in C and N freshwater cycles/budgets that require further research. Activity of water and sediment at the sediment-water interface is essential to understanding the transformation of the hydro biome (Daumas, 1990) and aquatic chemistry, but is difficult to analyze due to the coupled processes of organic matter oxidation, oxygen consumption, and nutrient cycling (Norlem et al., 2013). The microbial transformations of dissolved- and sediment-organic matter that change the biological and chemical signature of water and sediments has not been studied extensively (Davis and Fox, 2009), and requires further work to elucidate their (non)conservative nature and degradation rates. Previous laboratory-scale experiments on the mineralization of sediment organic matter have compared decomposition rates in both oxygenated and anoxic systems (Gale et al., 1992; Hulthe et al., 1998; Lehmann et al., 2002). These results suggest the microbial pathways in anaerobes

do not degrade organic matter as rapidly as aerobes, which is in agreement with basic knowledge in the energetics of aerobic and anaerobic respiration. Further, the Lehmann et al. (2002) study suggests the fate of organic matter under varied redox conditions is dependent on the chemical composition (i.e. organic carbon availability) of the decomposing organics.

With the research need in mind, our motivation was to estimate the reactivity and isotope enrichment of sediment and dissolved constituents in a laboratory incubation study, as well as compare the results qualitatively with the field measurements. To do so, we investigated the initial bio-availability characterization of fine sediments from the South Elkhorn Creek in the environmental laboratory. Two types of sediment are studied, including sediments representative of upland soil and in-stream bed sediments, separately incubated in open and closed systems. The study is designed to investigate biogeochemical transformations over a long-term incubation (4+ months). Sediment and water samples are analyzed to determine the elemental concentration of carbon and nitrogen, as well as isotopic ratios of oxygen, nitrogen, and carbon in order to (1) elucidate the fate of carbon and nutrients during elemental mineralization and spiraling as well as isotope fractionation, (2) investigate the role of biotic processes in transforming nitrogen and carbon, (3) and combine the data results with organic matter fate models (i.e., decay models) consistent with biogeochemical processes in the water sciences and engineering. Results of this study will be integrated with on-going research in the area of carbon and nutrient fate in stream systems.

1.1.2 Research Need #2

Conservativeness, or non-conservativeness, of carbon and nitrogen stable isotope tracers used to perform sediment fingerprinting during low, moderate, and high flow hydrologic events:

We find that very few studies exist focused on the conservativeness, or non-conservativeness, of carbon and nitrogen stable isotope tracers used to perform sediment fingerprinting during low, moderate, and high flow hydrologic events. Sediment fingerprinting studies often use carbon and nitrogen stable isotope values of sediment ($\delta^{13}C$, $\delta^{15}N$) as tracers. The fingerprinting studies almost always assume conservativeness of the stable isotope tracers as the sediment travels from its origin to the basin outlet. However, researchers rarely validate the conservative assumption given the methodological difficulty and cost in doing so. In this study, our motivation was to investigate the conservative assumption for carbon and nitrogen stable isotope values of sediment by focusing on potential isotope change during carbon oxidation and nitrogen mineralization, changes of isotopes due to fractionation of particle size and disaggregation during high flow events, and changes due to algae accrual during temporary instream residence.

Organic matter degradation is the biogeochemical change of sediment organic C, N to products including CO_2 *via* oxidation, NH_4 *via* mineralization, and more stabilized organic matter bi-products. Sediment organic matter degradation has the highest likelihood to occur in temporary in-stream sediment deposits. Shallow deposits can experience warm water conditions potentially promoting decomposition. Microbial mediated C and N isotope fractionation may accompany organic matter degradation and change $\delta^{13}C$ and $\delta^{15}N$ of the sediment source. Substantial changes to $\delta^{13}C$ and $\delta^{15}N$ could cause the tracers to be non-conservative.

"Algal stabilization is the coupled biotic-abiotic process by which algal biomass decomposes into more complex refractory carbon compounds in aquatic ecosystems for extended periods (Lara and Thomas, 1995; Leloup et al., 2013; Hotchkiss and Hall, 2015; Ford et al., 2017)" Algal stabilization results in refractory organic matter compounds being integrated to the sediment deposits known as the surficial fine grained laminae. The conservative assumption of $\delta^{13}C$ and $\delta^{15}N$ may be violation if algal stabilization is pronounced and the $\delta^{13}C$ and $\delta^{15}N$ of algae differs from that of the sediment source.

Disaggregation is the physical breaking up of sediment aggregates to water stable aggregates, clay-organic complexes, or individual grains as fluid processes cause slaking, raindrop impact and fluvial shear and tensile forces during transport (Hillel, 1980; Ghidey and Alberts, 1997; Droppo et al., 2005; Fox et al., 2014). Disaggregation has the potential to adjust the organic matter makeup of the sediment source, with one plausible change being the floating away of more buoyant organic dominated micro-aggregates that detach from the suspended sediment load. The physical process can impact the organic matter content of sediment collected for analyses during sediment fingerprinting. Changes in the organic matter to total sediment ratio in turn has the potential to cause non-conservativeness for $\delta^{13}C$ and $\delta^{15}N$, since the tracers un-mix carbon and nitrogen, respectively.

In this study, we investigate conservativeness, or lack thereof, associated with: (i) sediment organic matter mineralization by investigating isotope changes during laboratory incubation of fluvial sediment and isotope changes during low flow conditions over about two years of sampling in the field from two different sites; (ii) algal accrual by investigating samples collected during high flows versus low flows and assessing seasonal changes to

see if autochthonous matter becomes accrued in the sediment and changes its isotope signature; and (iii) disaggregation by inspecting longitudinal changes in fluvial sediment isotope signature during high flow events at upstream and downstream sites.

In our future research, our methods and results for tracer conservativeness will be combined with other analyses in the literature and previous research published by our group to write a journal paper. Our paper will center on assessing four potential changes the tracer signatures could undergo including, organic matter mineralization, algal stabilization, disaggregation, and the presence of a non-stationary source. We use laboratory experiments, field data, and numerical modelling to assess how each biogeochemical or physical process could change $\delta^{13}C$ and $\delta^{15}N$. We also will perform un-mixing simulations to see the relative importance of each process on sediment fingerprinting results.

1.2 Project Objectives

The objectives of this research were to:

1. Characterize the water quality of the stream water used in this study using measurements of dissolved carbon and nutrients.
2. Characterize the fluvial sediment used in this study using analyses of past data and measurements in this study.
3. Estimate biogeochemical reactions occurring and reaction rates for water and fluvial sediment using laboratory incubation and modelling.
4. Estimate isotope enrichment of sediment and water C and N occurring during biogeochemical reactions using laboratory incubation results and modelling.

5. Characterize fluvial sediment provenance and conservativeness during high flow and extreme flow hydrologic events using field measurements of C and N elemental and isotope values.
6. Characterize changes occurring to fluvial sediment organic matter during temporarily storage as well as tracer (non)conservativeness using field measurements of C and N elemental and isotope values.
7. Investigate field-based fluvial sediment agreement with laboratory incubation results by assessing seasonal and flow regime dependence of field measurements of C and N elemental and isotope values.

The project objectives provide the sub-headings of the Literature Review (Ch 2), the Methods (Ch 3), and the Modelling Results and Discussion section (Ch 5). Chapter 4 is Data Results and presents and discusses each data result from the incubation study. Chapter 6 is the Conclusion and answers each of the seven objectives.

Chapter 2 - Literature Review

2.1 Fluvial sediment in agricultural and urban streams

Agricultural and urban impacted streams are recognized to have excess nutrients that are exported to depositional zones and to coastal waters. “Transformations and cycling of nutrients such as ammonium and nitrate in streams are important factors in the overall export magnitudes from watersheds (Peterson et al., 2001; Mulholland et al., 2009; Rode et al., 2016; Clare, 2019).” A fluvial system, originally postulated by Vannote et al. (1980) as the River Continuum Concept, is a discrete network of streams transporting water and sediments from source to sink, distinguished by geomorphic zones. The three zones outlined by Schumm (1977) include a production, transfer, and deposition classification, each relating to varying longitudinal trends in channel morphology and sediment regimes. Fluvial networks not only transport sediment from headwaters to deposition zones, they also harbor diverse benthic ecosystems that can generate autochthonous carbon and decompose organic matter (Raymond et al., 2013).

The transfer zone reflects the transition between the production and deposition zone, as the traveling sediments are eroded, deposited, and reworked over various spatial and temporal scales (Tooth and Nanson, 2011). Equilibrium sediment exchange in the transfer zone is the simultaneous deposition of suspended sediments countered by equal erosion of sediment from the streambed. This process does not change the sediment load or storage, but does impact the biogeochemical composition of sediment organic matter (Mahoney et al., 2018). Lowland stream networks within agricultural and urban mixed land use areas are susceptible to efficiently store sediment that assimilate nutrients. A significant

occurrence of fluvial sediment storage in the streambed promotes chemical bonding of nutrients due to the cohesive nature of high surface area particles (Birgand et al., 2007).

2.2 Organic matter pools prevalent in fluvial sediment

I. Reactive pool

The reactive pool of SOM includes constituents that are readily available for decomposition. This includes heterotrophic biomass generated during cell growth, decomposable plant material that are nonlignified carbohydrates, and any algal biomass assimilated into the sediment.

II. Resistant pool

The resistant pool of the SOM includes material that is recalcitrant or not highly susceptible to decomposition. This includes resistant plant material that is lignified carbohydrates, or hemicellulose that is covalently bonded to lignin, and humified matter that has been through multiple stages of decomposition.

Sources of sediment organic matter (SOM) investigated in this study can be separated by origin as terrestrial-derived, or allochthonous, organic matter and autochthonous, or in-stream generated organic matter. The carbon and nitrogen composition of organic matter in sediments results from several complex processes including inputs from allochthonous sources, biosynthesis in the photic zone, and organic matter degradation and bacterial growth in the water column and in the sediment (Lehmann et al., 2002).

Allochthonous organic matter can originate from plant litter, soil organic matter and soil detritus. Soil organic matter that enters streams are a combination of fresh, labile plant material that has undergone a first stage of decomposition, as well as humified matter

that is resistant to further degradation. Terrestrial litter and litter derived SOM is readily accepted as a lower quality source of organic matter relative to algal carbon. Decomposition rates for terrestrial material have been shown to be orders of magnitude lower than that of in-stream derived carbon (Enriquez et al. 1993; Webster et al., 1999; Six and Jastrow, 2002). The lower quality of terrestrial-derived material may be due to the chemical composition including more recalcitrant carbon compounds, such as lignin and hemicellulose while algal biomass is composed primarily of highly labile polysaccharides such as glucose (Lane, 2013).

2.2.1 Plant Material

Hemicellulose, cellulose, and lignin are the three main components of plant biomass and they in general cover 20-40, 40-60, and 10-25 percent by weight for lignocellulose biomass (McKendry, 2002a; Yang et al., 2007). Hemicelluloses are short chain polysaccharides with 500-3,000 monomer units while celluloses are long chain polysaccharides made up of 7,000-15,000 glucose monomer units (Gibson, 2012). Broadly speaking, the cell walls of plants are made up of cellulose fibers reinforcing a matrix of hemicellulose and either lignin or pectin in one or more layers (Gibson, 2012). During the early stages of plant litter decomposition, cellulose is degraded preferentially yielding glucose which is readily assimilated and consumed in metabolism (Sinsabaugh & Follstad Shah, 2011). Cellulose does not contain N or P so decomposers also produce enzymes to acquire theses nutrients from other sources. The principal organic N sources are amino acids (peptides, proteins) and amino sugars (chitin, peptidoglycan) (Nannipieri & Eldor, 2009; Sinsabaugh & Follstad Shah, 2011). The empirical chemical structure of cellulose $(C_6H_{10}O_5)_n$ suggests that about 44% of the component consists of organic carbon.

Lignin is a complex polymer that fills the space in the cell wall between cellulose, hemicellulose, and pectin components in vascular tissues and is the predominant plant-inherited molecular structures in soils (Pengerud et al., 2017). The empirical structure of lignin $(C_{31}H_{34}O_{11})_n$ measured from aspen trees suggests 64% of the cells are comprised of organic carbon. There is an accepted range for the organic matter composition of plant litter in the literature with a mean C:N:P ratio of 3000:46:1 (C:N 65.2:1) (Reich and Oleksyn, 2004; Sinsabaugh et al., 2009) that corroborates with studies on temperate and tropical leafs that found a C:N ratio of 66.2 ± 6.3 (McGroddy et al., 2004; Cleveland and Liptzin, 2006).

When interpreting the chemical composition of plant organic matter (or soil organic matter) it is important to remember the composition has a wide range and is subject to change due to the decomposition continuum. Plant litter is degraded in a first stage as water soluble compounds and nonlignified carbohydrates are preferentially decomposed and their relative concentrations go down, whereas lignin decomposes little and its relative concentration goes up (Berg, 2000). The second stage begins when the only remaining carbohydrates are lignified and decompose in association with lignin, resulting in a stabilization of the lignin fraction. This is when decomposition becomes extremely slow as the remaining material becomes increasingly humified and recalcitrant (Berg, 2000).

2.2.2 Humified Material

Humic substances form from microorganisms breaking down plant and animal residues, which are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during decay (Battin et al., 2016). The humified material is a relatively stable component formed by humic acids, fulvic acids, and

humins (Tan, 2011). The C:N ratio of newly formed humified organic matter has been reported as C:N = 9.5 (Nicolardot et al., 2001), lower than what is measured in this study for fluvial sediment organic matter within small stream systems (10-12). The low organic matter content of humified material is due to the fresh labile sugars from plant material being fully degraded before assimilating with other particles. Humus combines with inorganic minerals (clays) to form organic-inorganic complexes in the aggregate, which are even further resistant to decomposition. (Bol, et. al., 2003).

2.2.3 Algal Material

Autochthonous material, or organic matter produced in-stream, can be viewed as carbon from benthic production of autotrophic algal biomass as well as heterotrophic biota that break down organic carbon for energy. Algae are a highly diverse group of photoautotrophic organisms with chlorophyll a and unicellular reproductive structures (Stevenson et al., 1996). Benthic algae are those that live on or in association with substrata. Phytoplankton are algae suspended in the water columns. Most benthic algae in freshwater habitats are blue-green algae, green algae, diatoms, or red algae. The blue-green algae, green algae, and diatoms have the greatest morphological; diversity with unicellular, colonial, and filamentous forms (Stevenson et al., 1996). Autotrophs including filamentous algae and diatoms colonize within the surface sediment (Battin et al., 2003; Garcia-Aragon et al., 2011).

During photosynthesis, autotrophs secrete extracellular polymeric substances or EPS. Algal EPS is primarily acid polysaccharides secreted from the cell membrane that act as a gluey substance and holds sediment particles together (Kies et al., 1996). The empirical chemical formula for algal biomass is $C_{106}H_{263}O_{110}N_{16}$ suggesting an organic carbon

content of 36%, nitrogen content 6.4%, and estimated C:N ratio of 5.68. These values are in agreement with literature derived values of C:N between 4 and 10 for algae (Meyers, 1994), and unicellular cellular algae such as phytoplankton and benthic microalgae following a Redfield ratio of C:N:P = 106:16:1 (C:N 6.63) (Baird and Middleton, 2004). Further, a decomposition incubation study found lacustrine diatoms to have a relatively consistent C:N ratio of 7.6 to 8.3 before and during decomposition (Lehmann et al., 2002). The quality of organic carbon in algal biomass has been found to differ from terrestrial derived fine sediment as lignin contents have been measured as nearly half in comparison to fine sediment (Yoshimura et al., 2008).

Heterotrophic bacteria are able to carry out the oxidation of organic material, and are considered the primary decomposers in the environment (Rittman & McCarty, 2001). Heterotrophic decomposition is the biological process by which carbon is converted from an organic state into an inorganic state. During the decomposition process carbon dioxide is released as well as energy, water, and nutrients (i.e. mineralization). The rate of decomposition is mainly a function of the type of soil organism, the physical environment, and the quality of organic matter (Brussaard, 1998). Macromolecules of nucleic acids, proteins, carbohydrates, and lipids make up the structure of the heterotrophic biomass (Madigan et al., 2008). N and P are further concentrated in soil microbial biomass, which has a mean C:N:P ratio of 60:7:1 (C:N 8.6:1) (Cleveland & Liptzin, 2007). Similar elemental ratios have been reported for heterotrophic microbial biomass associated with surface sediments of inland waters (Cross et al., 2005). The relationship between the generation via photoautotrophs and the decomposing heterotrophs creates a feedback driving the carbon cycling in stream biofilms (Battin et al., 2016). Phototrophs such as

diatoms, green algae and cyanobacteria (blue-green algae) exude organic compounds such as carbohydrates and amino acids, which are highly available to the heterotrophic metabolism (Haack & McFeters, 1982). Also the respiratory carbon dioxide from these heterotrophs can be assimilated by the phototrophs. This feedback loop is a product of the internal carbon cycling of biofilms in aquatic environments.

2.3 Biogeochemical reactions in stream water with fluvial sediment

2.3.1 Hydrolysis

Hydrolysis is a chemical reaction in which bonds are broken in a molecule due to a reaction with water. The initial breakdown of organic N compounds via hydrolysis releases soluble organic matter compounds available for subsequent mineralization (Harvey et al., 1995; Lehmann et al., 2002). Urea is the most commonly used N-source in synthetic fertilizers globally, and makes up a relatively labile component of organic nitrogen ubiquitous in soils and sediments (Sigurdarson et al., 2018). The enzyme urease catalyzes a reaction in which one molecule of urea is hydrolyzed to form two molecules of ammonia (NH_3) and one carbonic acid (H_2CO_3). The half-time of the urease-catalyzed reaction is only 20 ms at 25 °C (Callahan et al., 2005; Estiu and Merz, 2004).

$$CH_4N_2O + 2H_2O \xrightarrow{Urease} 2NH_3 + H_2CO_3$$

Urease is common in nature and is produced by a wide range of organisms. Urease has been found in several species of bacteria, fungi, algae, plants, and invertebrates (Bekheet and Syrett, 1977; Booth and Vishniac, 1987). Urease-producing microorganisms, known as ureolytic, are found in nearly all ecosystems, including soil (Sigurdarson et al., 2018).

2.3.2 Decomposition

Heterotrophic decomposition is the biological process by which organic matter is broken down into simpler organic substrates, while also releasing inorganic C and nutrients (mineralization). Decomposition of carbon atoms within an organic compound has been defined as the release of CO_2 from metabolizing organisms (Zibilske, 1994). In the presence of oxygen, the process termed aerobic respiration follows the reaction:

$$C_5H_7O_2N + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O$$

Where a generalized form of an organic material is oxidized and released as an inorganic form of carbon (carbon dioxide) and nitrogen (ammonia). In total, chemoorganotrophs oxidize organic matter to CO_2 and subsequently releases soluble inorganic nitrogen (NH_3), which is then available for further microbial processing such as chemolithotrophic oxidation to nitrate via nitrification. The mineralization of organic matter during cell respiration is driven by oxidative phosphorylation, or the generation of energy (ATP) from cycling of electrons which produces a proton motive force (Madigan et al., 2008).

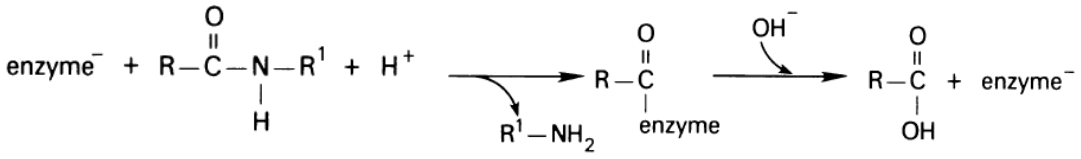
2.3.3 Mineralization

Mineralization is the oxidation of nutrients within organic matter released as soluble inorganic compounds, a process mediated by respiring microorganisms. The production of nitrate by mineralization of organic nitrogen can be represented in the following steps:

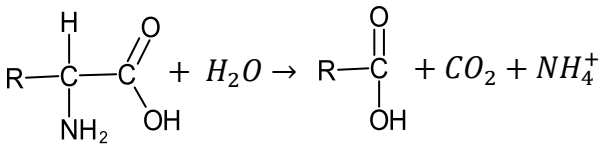
$$org\ N \overset{1}{\rightarrow} NH_4^+ \overset{2}{\rightarrow} NO_2^- \overset{3}{\rightarrow} NO_3^-$$

Nitrogen mineralization is the two-step process of ammonification (org-N to NH_4^+), then nitrification (NH_4^+ to NO_2^-, then NO_3^-). The first step in the mineralization process is the enzymatic conversion of organic nitrogen to ammonium termed ammonification, which is carried out exclusively by heterotrophic microorganisms that utilize organic C substances as an energy source (Benbi and Richter, 2002). The ammonification reaction begins when a peptide bond undergoes hydrolysis, in which a basic group of an enzyme becomes bonded to a C atom of the CO group in a chain of amino acids (Ladd and Jackson, 1982). As the reaction occurs, the N atom within the peptide bond is displaced and subsequently receives a proton donated by an acid group of the enzyme or from water (Ladd and Jackson, 1982).

Diagrams adopted from Ladd and Jackson, 1982



The displaced N atom continue to bond with free H^+ ions and undergoes further hydrolysis until amino acid groups are formed (Ladd and Paul, 1973; Ladd and Jackson, 1982). Then chemical reactions of amino groups (NH_2) associated with the original organic form are converted into ammonia (NH_3), or its ionic form ammonium (NH_4^+) (Strock, 2008). The microorganisms responsible for this process also utilize both C and N during growth to build-up microbial biomass (Benbi and Richter, 2002). The generalized reaction for ammonification of soil organic compounds is as follows (Strock, 2008):



The complex organic compounds deamination of its amino group results in a simpler organic compound (i.e. carboxylic acid) (Krebs, 1935) coupled with the release of carbon dioxide and the newly formed N-compound (i.e. NH_4^+) (Strock, 2008). The ammonification of organic nitrogen is the intermediate step in mineralization in which the generated form of N can then be further used by ammonium oxidizing bacteria (AOB).

2.3.4 Nitrification

Nitrification is the biological oxidation of ammonia (NH_3) or ammonium (NH_4^+) to nitrite (NO_2^-) followed by the oxidation of nitrite to nitrate (NO_3^-) (Sahrawat, 2008). Nitrifying bacteria are autotrophs, chemolithotrophs, and obligate aerobes (Rittman and McCarty, 2001). Nitrifiers utilize CO_2 as their sole source of cell carbon, obtain energy (ATP) from oxidizing inorganic compounds, and require oxygen for respiration. During nitrification, N atoms originate from the source of oxidized material (i.e. NH_4^+, NO_2^-), while O atoms originate from O_2 and H_2O (Kendall et al., 2007). It has been proven (i.e. Winogradsky) that nitrifying bacteria can use CO_2 as the sole carbon source and ammonia as the sole electron donor (Madigan et al., 2008). The pathway of CO_2 fixation to organic matter follows the biochemical steps of the Calvin Cycle, similar to other autotrophic bacteria in the environment.

Autotrophic AOB (commonly *Nitrosomonas)* convert ammonia and ammonium to nitrite by the following reaction:

$$NH_4^+ + \frac{3}{2}O_2 \overset{6e}{\rightarrow} NO_2^- + H_2O + 2H^+$$

Where the process has a couple of intermediate steps before forming nitrite. Respiring cells utilize O_2 as a direct reactant for the initial monooxygenation of NH_3 to the intermediate NH_2OH (hydroxylamine) (Rittman et al., 2001). The key enzyme Ammonia monooxygenase is a membrane bound protein that forms hydroxylamine from the oxidation of ammonia (Madigan et al., 2008). The oxidation of NH_2OH to NO_2^- is carried out by the enzyme hydroxylamine oxidoreductase, where release of two electrons from the dehydrogenation of NH_2OH is coupled to the synthesis of ATP during the formation of NOH (Schmidt, 1982). Oxidation of NOH to NO_2^- occurs with the release of 4 electrons and the net addition of an atom of oxygen derived from O_2 (Schmidt, 1982).

Next, nitrite oxidizing bacteria (commonly *Nitrobacter)* finish the conversion of nitrite to nitrate (Sahrawat, 2008).

$$NO_2^- + \frac{1}{2}O_2 \xrightarrow{2e} NO_3^-$$

The reaction is carried out by the enzyme Nitrite oxidoreductase, which catalyzes the oxidation of nitrite coupled to the reduction of oxygen, resulting in the generation of ATP (Madigan et al., 2008). Nitrification is often limited by oxidation of ammonia, and nitrite rarely accumulates in most environments (Prosser, 2007).

The overall nitrification process is represented by the following equation:

$$NH_4^+ + 2O_2 \xrightarrow{8e} NO_3^- + H_2O + 2H^+$$

Several studies have indicated that the ammonification process involves little isotopic fractionation($\varepsilon_{NH_4^+ - org\ N} \approx \pm 2‰$), but the nitrification step has a large kinetic

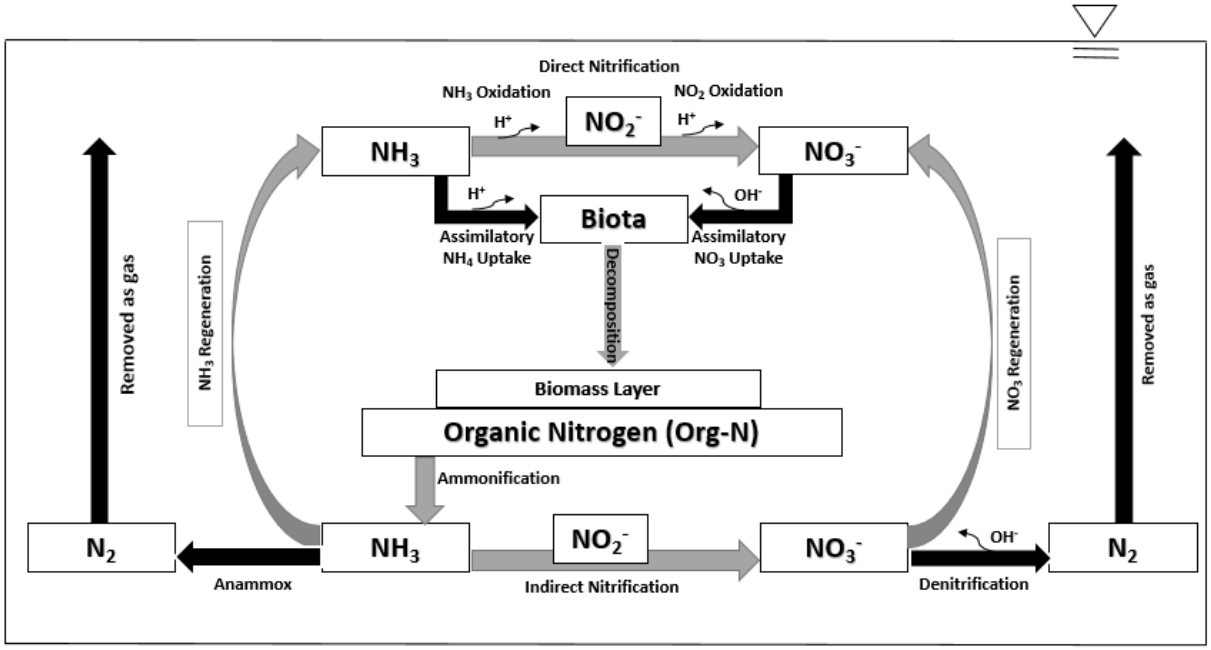
fractionation effect($\varepsilon_{NO_3^- - NH_4^+} \approx -35\ to + 5‰$) (Delwiche and Steyn, 1970; Miyake, 1971; Freyer and Aly, 1975; Mariotti et al., 1981, Casciotti et al., 2003; Kendall et al., 2007). It has been recognized that the overall fractionation for the mineralization process depends on whether ammonification or nitrification is the rate-limiting step (Feigin et al., 1974; Freyer and Aly, 1975; Mariotti et al., 1981).

2.3.5 Mineralization and Isotope Fractionation

If a relatively large amount of ammonium is available, the mineralization process is limited by the nitrification step. The generated nitrate is then strongly depleted in ^{15}N, and will continue to have low $\delta^{15}N$ values if ammonium is present and readily available (Heaton, 1986). However, most of the mineralizable organic nitrogen in soils is slowly converted to ammonium. When little ammonium is available the mineralization process is limited by non-fractionating ammonium oxidation, and the nitrate will tend to have an isotopic signature similar to that of organic nitrogen (Heaton, 1986). The situation where ammonification is the rate-limiting step has been shown to dominate in field environment and laboratory incubation soil studies (Delwiche and Steyn, 1970; Mariotti et al., 1981; Mayer et al., 2001).

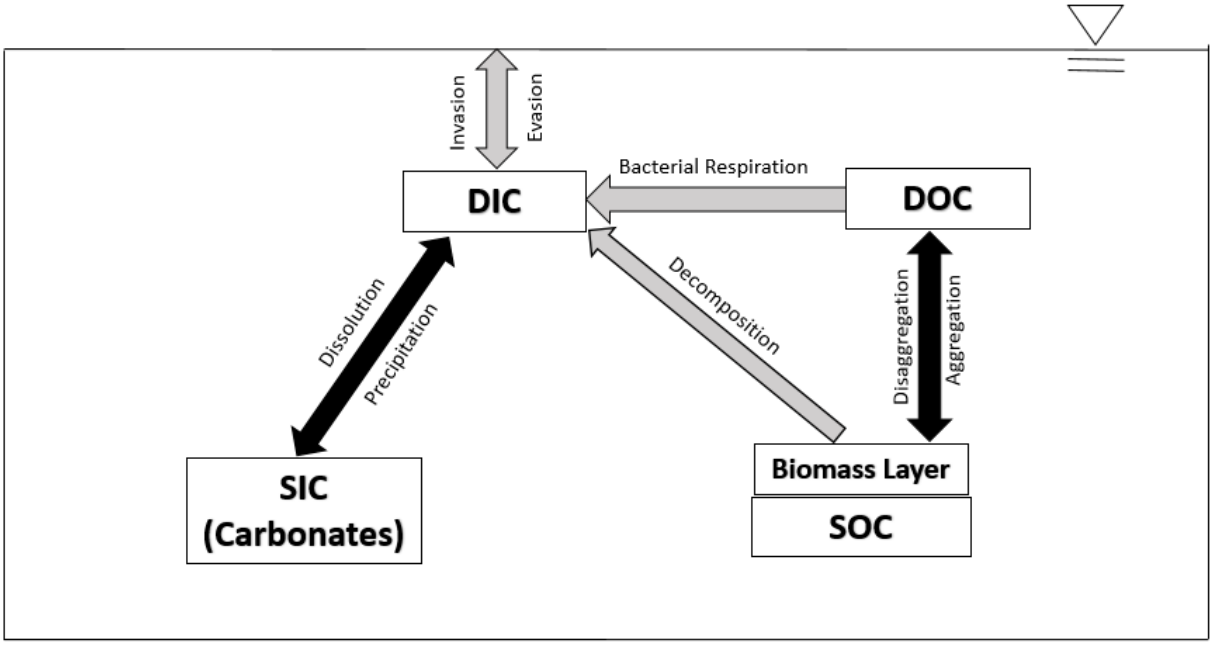
In general, the lighter isotope (the one with the lower mass) reacts faster, resulting in products that are isotopically lighter (i.e., have fewer neutrons) than the reactants. When microbes convert ammonium to nitrate (nitrification), the nitrate being formed is isotopically lighter (lower $\delta^{15}N$ value) than the ammonium being left behind (Kendall and Aravena, 2000). It has been reported in literature the $\delta^{15}N_{NH4}$ of soil is within a few permil of the $\delta^{15}N$ of total organic N in the sediment due to minimal isotope fractionation (Kendall et al., 2007).

Figure 2-1: Conceptual model of nitrogen cycling in streams



Gray arrows denote processes expected to affect N cycling; black arrows are not expected to have substantial impact on N cycling in flasks (i.e. minimal activity). Modified from Peterson et al., 2001; Ford et al., 2017; Sigurdarson et al., 2018.

Figure 2-2: Conceptual model of carbon cycling in streams



Gray arrows denote processes expected to affect C cycling; black arrows are not expected to have substantial impact on C cycling in flasks (i.e. minimal activity). Modified from Ford et al., 2015

Chapter 3 – Methods

3.1 Collect and analyze stream water

Collect and analyze stream water potentially characteristic of agricultural- and urban-impacted stream systems that are nitrogen-limited:

We collected and analyzed stream water from the South Elkhorn Creek. We collected this water because (i) this was our study site focused on in research, such as for the sediment lab and field components; and (ii) based on our past research, we expected water quality to be characteristic of agricultural- and urban-impacted-streams that are nitrogen limited.

The South Elkhorn Creek (see Figure 3-1) drains a mixed land use watershed including primarily agricultural pasture, urban/suburban region of southwestern Lexington, Kentucky, and small sections of row crops (< 2%) (See Clare, 2019 for full watershed characterization). The creek itself is a lowland stream network with an efficiency to store sediments that assimilate nutrients during sedimentation. Moderate and high flows in lowland catchments transport a heterogeneous mixture of upland, bank, and, streambed fine particulate organic matter from autochthonous and terrestrial sources. The South Elkhorn Creek has been chosen as the study site because of a plethora of historic information and on-going research in stream sediment transport and biogeochemistry. Additionally, the study watershed's significant occurrence of fine sediment storage in the streambed promotes chemical bonding of nutrients due to the cohesive nature of high surface area sediments.

We characterized the water quality from the South Elkhorn Creek with measurements, and then the water was later used as the incubation study's medium with

the intent that the natural microbial community would facilitate microbially-mediated oxidation and nitrification. We collected water from the creek on April 6th, 2019 during a low flow period with a peak discharge of 1.25 m^3/s. Water was collected in two 5-gallon buckets and stored at 4°C in the laboratory. The water was filtered with a #270 mesh to remove coarse particles (<53 μm) that may contribute to the incubated sediment pool, while also retaining the natural microbial population of South Elkhorn creek water. The water was then refrigerated until analyses of initial conditions and incubation. The dissolved constituents of the water, including DIC, DOC, NO_3^-, NH_4^+ & TKN, and the isotope signature of the water, including $\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$, & $\delta^{13}C_{DIC}$, were analyzed. The methods for analyses are described in Sections 3.3, 3.4 and in the Appendices.

We did not study phosphorus directly in this study, however, many previous measurements have shown that this system is not P limited. Yearly averages of orthophosphate as phosphorous (PO_4^--P) were 0.216-0.359 mg l^{-1}, and a total yearly watershed average of 0.244 mg l^{-1} (Clare, 2019).

3.2 Collect and analyze fluvial sediment

Collect and analyze fluvial sediment characteristic of terrestrial-derived soil organic matter and aquatic-derived, algal organic matter:

Our intent was to collect two different types of fluvial sediment including sediment dominated by terrestrial-derived soil organic matter and fluvial sediment that was a mixture of terrestrial- and aquatic-derived organic matter. We chose these two types of fluvial sediment to focus on because we hypothesized that the two types were representative of fluvial sediment reported in the literature, more broadly. For example, terrestrial dominated sediment reflects sediment transported in extreme events in mixed-use

catchments or sediment transported in steep catchments with no fluvial storage; and a mixture of terrestrial- and aquatic-derived sediment reflects sediment transported during low and moderate hydrologic events in low and moderate gradient mixed-use catchments with fluvial storage. We termed the sediment types as: 1) "upland sediment" transported from the hillslopes of the watershed during extreme rainfall events; and 2) "in-stream sediment" relatively high carbon bed sediments that reflect benthic autotrophy are investigated.

Upland and in-stream sediment samples were collected at Ramsey's located at the mid-point of the watershed (~30 km^2) and draining the upper catchment; and at Gage located at the watershed outlet (62 km^2) and draining both the upper and lower catchment. A sediment trap sampling regime that emphasizes transported sediments to isolate the upland and in-stream end members rather than sediments collected immediately at the source locations for the following reasons: (i) Transported sediments have undergone some disaggregation due to fluvial shear stress that would make them more typical of sediments as opposed to upland soils (i.e., fine sediments are well known to experience size fractionalization/sorting during transport from the soil source to the stream, Collins et al., 1997). (ii) Transported sediments provide an integrated signal that account for spatial variability, rather than collecting a sediment source from a single point or set of points in the watershed.

Upland sediments are expected to be a mixture of surface, gully and bank sediments (i.e., from tributary banks). However, we treat these sub-sources as one lump end-member that is called "upland sediment" (and sediment organic matter) that is delivered laterally to the stream corridor. We have attempted to isolate transported upland sediments by

choosing transported stream sediment samples that were collected from the Gage location draining the entire watershed during extreme rainfall events, or just after extreme rainfall events. The extreme rainfall events are known to produce pronounced sediment connectivity between the uplands and the stream corridor for the South Elkhorn system (Mahoney, 2017), and thus high contributions of upland sediments was expected within the transported sediment load.

We qualify that stream sediments will always likely be some mixture of sediment from upland and in-stream sources, however, the chosen samples from extreme events that will be expected to have the greatest contribution of upland sediments relative to the other sediments mentioned below (Mahoney et al., 2018). The upland sediment samples relate to very high peak flows and corresponding very high sediment mass collected in the sediment traps (i.e., Phillips/Walling tubes, Phillips et al., 2000) and archived in the laboratory. The extreme event occurring in September 2006 was the highest streamflow event on record for the past 14 years. We also have found that upland sediments had relatively low SOC and SN and high $\delta^{13}C$ and $\delta^{15}N$ values, which correspond well with surface and subsurface soil samples from hay agriculture known to dominate the basin.

In-stream bed sediments with high carbon content reflecting benthic autotrophy is a mixture of bed sediment that has received pronounced contribution from the growth and decomposition of algae and other periphyton within the sediment. This sediment source tends to be transported in the stream channel during low and moderate flow events during late fall and early winter because the sediment has had the time to accumulate labile algae organic matter that has gone through a first stage of decomposition. Based on analyses of historic data and multi-year time series decomposition (i.e., empirical mode decomposition

reported in Ford et al., 2015), the 2008-2009 late fall and winter sediments are the clearest picture of an in-stream bed sediments with relatively high autochthonous carbon accrual.

When selecting the bed source samples, we tried to avoid using transported bed sediments that were likely impacted by high flow events in the months prior to sample collection because these samples would contain a high contribution of recently deposited upland sediment. These criteria negated using many of the samples collected from the past 13 years. We qualify that the transported sediments will always be a mixture of bed sediments and some previously deposited sediments from the uplands; however, the 2008-09 fall-winter sediments had a very low occurrence of high flow events and streamflow was always less than 400 ft^3/s (11.3 m^3/s) at the watershed outlet (i.e., Gage location) from late April 2008 to the time of sampling in October 2008. In addition, numerical model results of the stream benthic processes showed that the modeling results from Fall 2008 matched very well with the transported sediments during this time period (Ford and Fox, 2014); therefore, we feel most confident that the low-moderate flows during this Fall 2008 and winter 2009-time period likely included primarily the fluffy newly generated SFGL (as opposed to a larger proportion of upland sediment deposits).

The fall 2008 and winter 2009 sediment samples that reflect the in-stream bed sediment accrual of autochthonous carbon were found to have relatively high SOC and SN and low $\delta^{13}C$ and $\delta^{15}N$. The sediments were collected during low to moderate streamflow events. We also find that the statistically significant, quasi-seasonal intrinsic mode functions for FPOC (in the top plot of figure 7 in Ford et al., 2015) show that this time period has high levels of carbon content within the sediments. Since this seasonal-mean

curve is based on data, it helps guide us to highlight that carbon content is in fact "high" for the stream, reflecting autotrophic input, relative to the rest of the year.

3.3 Laboratory Experiments

Perform laboratory incubation to provide data for estimating biogeochemical reactions occurring, reaction rates, and isotope enrichment occurring during biogeochemical reactions (including carbon oxidation, CO_2 evasion, nitrogen mineralization and nitrification):

The incubation batch experiments performed aim to simulate microbial degradation during transformation at the sediment-water interface, while investigating temporal variations in organic matter (OM) of fine sediments under differing redox conditions. Each experimental system was incubated for 140 days in 250 mL Erlenmeyer flasks on an orbital shaker (50 rpm) platform in a dark, temperature-controlled (25°C) environment. South Elkhorn Creek surface water containing a natural microbial community was used as incubation medium for the experimental systems. Transported upland and in-stream sediments with distinct organic signatures served as the enzymatic substrate of this study, due to interest in examining SOC turnover, nutrient fate, and carbon availability on biogeochemical processing. Upland sediment, terrestrial material delivered laterally to the stream corridor during extreme rainfall events, reflect relatively low SOC and SN and high $\delta^{13}C_{Sed}$ and $\delta^{15}N_{Sed}$. In-stream sediment, qualified as bed material induced by benthic autotrophy, accumulates labile organic matter during extended low flow periods, and reflect relatively high SOC and SN and low $\delta^{13}C_{Sed}$ and $\delta^{15}N_{Sed}$.

The laboratory incubation study was designed to characterize the initial bioavailability of fine stream sediments and examine isotope fractionation during oxidation. The alteration of the elemental (SOC & SN) and isotopic ($\delta^{13}C_{sed}$ & $\delta^{15}N_{sed}$)

signature was observed in a series of two sets of batch experiments to investigate the decomposition kinetics of SOM in open and closed systems. An autoclaved system was also investigated to elucidate abiotic transformations potentially impacting the fate of organic carbon and nutrients. Sediments representative of upland (or allochthonous) derived materials and in-stream sediment reflecting benthic autotrophic (or autochthonous) transformations were incubated separately in each system. The dissolved constituents (DIC, DOC, NO_3^-, NH_4^+ & TKN) and isotopes ($\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$, & $\delta^{13}C_{DIC}$) in each experiment were also analyzed to examine sediment and water exchange.

Each system (flask) is subjected to a sample code corresponding to the experimental conditions as follows:

- Sample I.D.: (Condition) (Type of sediment) - (Sampling Period) (Replicate)
- *Conditions:*
    - Oxic (O): open system with freshwater as incubation medium supporting aerobic activity.
    - Hypoxic (A): closed system with low oxygen (purged with N_2 gas) freshwater as incubation medium.
    - Control (C): sterilized system using autoclave (abiotic control).
- *Sediment:*
    - Upland (U): fine sediment transported from gullies and rills of hillslopes during extreme rainfall events.
    - In-stream (I): fine bed sediment with relatively high carbon content that reflect benthic autotrophy.
    - Blank (B): no sediment within incubation medium (blank control).

Sample Code Examples: For example, experimental system with upland sediment in an open system collected during the 3rd sampling period. The replicates of this system were pooled for analyses. (i.e. OU-3). Experimental abiotic control system with instream

sediment in open conditions collected during the 3rd control system sampling period. The replicates of this system were analyzed separately. (i.e. OIC-3a)

Study Preparation: Archived samples stored in the University of Kentucky's Hydro Systems Laboratory were pooled together with the intent to categorize sediments that were representative of terrestrial or autochthonous source material. Upland sediments were selected from samples collected during extreme rainfall events and in-stream bed sediments were selected from samples collected during extended periods of base flow, see sample selection rationale for further details. Subsamples of the respective sediments were sieved using a 53-micron mesh and deionized (DI) water. The separated water and fine particulates were collected in a pan and transferred to conical bottles for centrifugation, then decanted to remove excess water.

Frozen sediment samples were then lyophilized to remove any remaining water, resulting in a dried sediment sample to be used for incubation. The sediments for each sample were then pooled and ground lightly using a mortar and pestle to ensure particle homogeneity. The samples were labeled and stored until incubation.

Water collected from South Elkhorn Creek was used as the study's medium. The water is filtered with a #270 mesh to remove coarse particles (<53 μm) that may contribute to the incubated sediment pool, while also retaining the natural microbial population of South Elkhorn creek water. The water was then refrigerated until incubation. A detailed procedure for the study preparation is included as appendix B.

Batch Incubation Setup: Erlenmeyer flasks (250-mL) were set up on an orbital shaker platform in a temperature controlled (25°C) environment. Flasks were filled with 150 mg

of sediment and 200 mL of South Elkhorn Creek surface water using a funnel and pipette. Open flasks were covered with cotton to limit contamination and remain open to the atmosphere, while closed flasks were purged with nitrogen gas for eight minutes and capped with a rubber stopper. Dissolved oxygen and pH measurements were recorded throughout the experimentation.

Abiotic Control: A series of open and closed flasks were prepared than sterilized using an autoclave to inhibit biotic activity. The control systems were triplicated and periodically sampled throughout the 140 day study.

Blanks and Replicates: Flasks with only creek water were sampled periodically for both the open and closed systems. The dissolved constituents were analyzed and serve as a control condition in comparison to our experimental systems. Each experimental system was triplicated to reduce sampling and analysis variability.

Periodic Sampling Routine: A series of each experimental system were periodically sampled twice for the initial 7 days due to the hypothesis of an initial rapid degradation to occur once the organic substrate is first introduced to water. Samples were then collected at weekly intervals for the next 21 days, and biweekly for the remaining 42 days of incubation. A 140-day incubation of each system was monitored to investigate long-term processes that may impact our systems. Flasks of the experimental systems were removed from the controlled-environment and measured for DO and pH. The contents of the flasks were transferred into pre-cleaned septa vials in preparation for separation. Water was siphoned with a syringe and filtered using Whatman Glass Fiber 0.45µm, 47mm filters, then separated into their respective splits for analyses. The remaining sediment sample was

transferred to a Falcon® 300 mL conical bottle, then frozen and lyophilized to remove any remaining water. The dried sediment samples were ground to a fine powder using a Wig-L-Bug mixer and transported to culture tubes until IRMS analysis.

Analyses Preparation: Sediment and water samples were prepared for analysis by the following steps.

Sample Pooling: Replicates of each incubated system were pooled together prior to analyses. Periodic triplicates of the pooled samples were run for analyses in order to get a standard error associated with the overall method.

Sediment Preparation: Powdered samples were weighed into tin capsules and acidified repeatedly with a weak (0.5 M) hydrochloric acid to remove inorganic carbon (carbonate) material. Inorganic carbon (IC) cannot be completely combusted at normal EA operating temperatures, and typically has a carbon isotope composition higher than organic carbon (OC), so if not removed the measured carbon isotope composition of the sample will be skewed towards that of partially combusted IC (Dabundo & Munizzi, 2018). All dried subsamples loaded into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analyses were conducted using an isotope ratio mass spectrometer (Thermo Finnigan Delta PLUS XP) interfaced with a Costech 4010 elemental analyzer. Average standard deviations for the samples of the elemental standard (acetanilide) were 0.82% and 0.11% for %C and %N, respectively.

Water samples were split into the following for analysis:

Nitrate, Ammonium, Dissolved Inorganic Carbon- Filtered samples were poured into pre-cleaned 40 mL VOC Sterile Septum Vials without acid preservation (see KGS 9056 and

KGS D515/ASTM D515). Samples were then refrigerated to 4°C and had a holding time of 28 days. Minimum of 25 mL per analysis.

Dissolved Organic Carbon- Filtered samples were poured into pre-cleaned 40 mL VOC Sterile Septum Vials and preserved with phosphoric acid. DOC vial with septa is required to inhibit air exchange. Samples were then refrigerated to 4°C. Minimum of 40 mL per analysis.

Total Kjeldahl Nitrogen- Filtered samples were poured into pre-cleaned 40 mL VOC Sterile Septum Vials and preserved with sulfuric acid. Samples were then refrigerated to 4°C. Minimum of 25 mL per analysis.

$\delta^{15}N/\delta^{18}O$ of Nitrate- Filtered Nitrate samples were poured into pre-cleaned 40 mL VOC Sterile Septum Vials without acid preservation (USGS RSIL, 2003a). Samples were then refrigerated to 4^0C and have a holding time of 4 weeks.

$\delta^{13}C$ of DIC- Filtered DIC samples were poured into pre-cleaned 40 mL VOC Sterile Septum Vials without acid preservation (USGS RSIL, 2003a). Samples were then refrigerated to 4^0C and have a holding time of 4 weeks.

Sample Storage: Water samples collected into pre-cleaned 40 mL VOC Sterile Septum Vials were stored in the dark and refrigerated to 4^0C for no longer than 2 weeks until sent for analyses.

Sample Delivery: Samples analyzed at the Kentucky Geological Survey or UK Stable Isotope Lab were carried by Brenden Riddle, or an undergraduate assistant. Samples sent to the Arkansas Stable Isotope Lab, water samples were shipped in insulated containers

with ice packs (to keep samples cooled to 4ºC) biweekly after sample collection. Samples were shipped overnight using UPS. Sample delivery groups (SDGs) of 20 or less were used (EPA-505-B-04-900A). Chain of custody forms were used to denote when samples are shipped and received. No hazardous materials were shipped during the course of this project.

Sample Custody: To document sample handling, the following procedure were used for chain of custody, and the Chain of Custody Form is included as Appendix A.

1. Person collecting samples completed the respective Field book log.
2. Person relinquishing packaged samples to carrier sign Chain-of-Custody form and obtain signature of the representative of the carrier.
3. Transported package included a copy of the Samples Collection Log and the Chain-of-Custody form.
4. Person receiving transported samples obtained signature of representative of carrier and sign Chain-of-Custody form.
5. Laboratory personnel completed Chain-of-Custody form to acknowledge receipt of samples.
6. Laboratory personnel signed Chain-of-Custody form when samples are disposed.
7. The Database Manager kept a copy of the Chain-of-Custody form.

3.4 Numerical Modelling

Perform kinetic and isotope modelling to estimate biogeochemical reaction rates and isotope enrichment during reactions (including carbon oxidation, CO_2 evasion, nitrogen mineralization and nitrification):

Kinetic rates for the oxidation of organic carbon and nitrification were computed using a first-order mass balance model. The first-order mass balances were used in the formulation of Rayleigh-like equations for the estimation of enrichment rates. Rates for the oxidation of organic carbon are split into two pools based on their reactivity to degradation. The model is manually calibrated such that modeled results are within best agreement with observed data in the laboratory. Parameter description and model terms are reported in Table 3.7. The modeled first-order rate constants are also reported in Table 3.7.

The contribution of fluvial sediment from each source is estimated using an unmixing model. The general equation of unmixing models is a mass balance

$$z^T = \sum_k (x_k^T \times P_k) \quad (1)$$

$$\sum_k P_k = 1 \quad (2)$$

where, z is the tracer data from the sediment sampled, x is the tracer data of the source, T represents the tracer being used, k indicated the pool source, and P is the fraction of a particular source.

$$Sed^{C/N} = \sum_k \left(x_k^{C/N} \times P_k\right) = \left(x_1^{C/N} \times P_1\right) + \left(x_2^{C/N} \times P_2\right) \quad (3)$$

An estimated C/N ratio for each source pool of organic matter is used as the tracer data in order to calculate the fraction of each particular source.

Initial Sediment Carbon and Nitrogen

The initial C/N ratio of the sediment incubated is measured and recorded as the starting point of the unmixing model.

$$Sed\frac{C}{N}^0 = \left(P_{alg}^0 * C/N_{alg}\right) + (P_{soil}^0 * C/N_{soil}) \quad (4)$$

The C/N data for the sources of algae and soil were determined based on values reported in the literature (Meyers, 1994; Baird and Middleton, 2004).

$C/N_{alg} = 6$ (Algal Matter)

$C/N_{soil} = 13$ (Surface soil) Used for terrestrial source of in-stream sediment.

$C/N_{soil} = 11$ (Subsurface soil) Used for terrestrial source of upland sediment.

The unmixing model is used to determine the initial contributions of each pool based on the tracer C/N data.

The initial amount of C in each pool is calculated by the product of the total C measured in the sediment and the initial fraction of a particular source.

$$SOC_{alg}^0 = P_{alg}^0 * SOC_T^0 \quad (5)$$

$$SOC_{soil}^0 = P_{soil}^0 * SOC_T^0$$

The initial amount of N in each pool is calculated by dividing the amount of OC from the previous step by the C/N ratio of each pool.

$$SN_{alg}^0 = \frac{SOC_{alg}^0}{C/N_{alg}} \quad (6)$$

$$SN_{soil}^0 = \frac{SOC_{soil}^0}{C/N_{soil}}$$

Initial DOM Carbon and Nitrogen

The initial C/N ratio of the DOM incubated is measured and recorded as the starting point of the unmixing model.

$$DOM\frac{C}{N}^{0} = (P_{rea}^{0} * C/N_{rea}) + (P_{res}^{0} * C/N_{res}) \quad (7)$$

The C/N data for the sources of reactive material and resistant material were determined based on values reported in the literature (Reich and Oleksyn, 2004; McGroddy et al., 2004; Cleveland and Liptzin, 2007; Sinsabaugh, 2009).

$C/N_{rea} = 50$ (Reactive Material)

$C/N_{res} = 10$ (Resistant Material)

The unmixing model is used to determine the initial contributions of each pool based on the tracer C/N data.

The initial amount of C in each pool is calculated by the product of the total C measured in the sediment and the initial fraction of a particular source.

$$DOC_{rea}^{0} = P_{rea}^{0} * DOC_{T}^{0} \quad (8)$$

$$DOC_{res}^{0} = P_{res}^{0} * DOC_{T}^{0}$$

The initial amount of N in each pool is calculated by dividing the amount of OC from the previous step by the C/N ratio of the pool.

$$DON_{rea}^{0} = \frac{DOC_{rea}^{0}}{C/N_{rea}} \quad (9)$$

$$DON_{res}^{0} = \frac{DOC_{res}^{0}}{C/N_{res}}$$

Sediment C and N at daily time steps and first order rate processes

A mass balance approach was applied to model biochemical processes within the incubations. Each pool of fluvial sediment was subjected to a kinetic expression to account for the amount lost at daily intervals. For the algal pool of organic matter, the amount of sediment organic carbon at each time step, SOC_{alg}^{i}, was given as

$$SOC_{alg}^{i} = SOC_{alg}^{i-1} - DEC_{alg}^{i} \quad (10)$$

where, SOC_{alg}^{i-1} is the mass of algal organic carbon from the previous time step (mg), and DEC_{alg}^{i} is the amount of OC from the algal pool decomposed to DIC (mg). Processes occurring within the flask are modelled based on a mass balance approach that utilizes first order kinetic expressions. All kinetic subroutines are coupled to a modified Arrhenius expression for temperature.

The first-order subroutine is defined as a process that depends on the amount of a given constituent, and is represented as a rate constant (k) according to first-order kinetics:

$$\frac{dM}{dt} = -kM \quad (11)$$

Integration of this equation yields: $$M_i = M_{i-1}e^{-k\Delta t} \times \theta^{\left(T_{(i)}-T_{ref}\right)} \quad (12)$$

With M_i representing the amount processed (mg), M_{i-1} the amount processing in the previous time step, k is the first-order rate constant (day^{-1}), θ is the temperature coefficient

(equal to 1.08) T in reference to measured temperature (°C), and Δt as change in time (days).

$$SOC_{alg}^{i} = SOC_{alg}^{i-1} \exp(-k_{alg}\Delta t) \times \theta_{dec}^{(T_{(i)}-T_{ref})} \quad (13)$$

$$SOC_{alg}^{i} - SOC_{alg}^{i-1} = DEC_{alg}^{i} \quad (14)$$

The mineralization of organic nitrogen is coupled to the decomposition of organic carbon via the C/N ratio of each source pool, as others have done in previous soil modeling (see Manzoni and Porporato, 2009).

$$MIN_{alg}^{i} = \frac{DEC_{alg}^{i}}{C/N_{alg}} \quad (15)$$

Also the amount of N within a pool at time step i is calculated using the relationship between the C/N ratio of each pool.

$$SN_{alg}^{i} = \frac{SOC_{alg}^{i}}{C/N_{alg}} \quad (16)$$

The more humified soil pool of the sediment follows the same set of equations.

$$SOC_{soil}^{i} = SOC_{soil}^{i-1} - DEC_{soil}^{i}$$

$$SN_{soil}^{i} = SN_{soil}^{i-1} - MIN_{soil}^{i}$$

$$SOC_{soil}^{i} = SOC_{soil}^{i-1} \exp(-k_{soil}\Delta t) \times \theta_{dec}^{(T_{(i)}-T_{ref})}$$

$$SOC_{soil}^{i} - SOC_{soil}^{i-1} = DEC_{soil}^{i}$$

$$MIN_{soil}^{i} = \frac{DEC_{soil}^{i}}{C/N_{soil}}$$

$$SN_{soil}^{i} = \frac{SOC_{soil}^{i}}{C/N_{soil}}$$

The total amount of sediment organic carbon and nitrogen is the summation of each pool at the desired time step (i).

$$SOC_{T}^{i} = SOC_{alg}^{i} + SOC_{soil}^{i} \quad (17)$$

$$SN_{T}^{i} = SN_{alg}^{i} + SN_{soil}^{i}$$

The contribution from each pool at a specific time step and the C/N ratio of the sediment was calculated as follows.

$$P_{alg}^{i} = \frac{SOC_{alg}^{i}}{SOC_{T}^{i}}$$

$$P_{soil}^{i} = \frac{SOC_{soil}^{i}}{SOC_{T}^{i}}$$

$$Sed\frac{C}{N}^{i} = \left(P_{alg}^{i} * C/N_{alg}\right) + \left(P_{soil}^{i} * C/N_{soil}\right) \quad (18)$$

DOM C and N at daily time steps and first order rate processes

The same mass balance approach was applied to model dissolved organic matter and its biochemical processes within the incubations. Each pool of DOM was subjected to a kinetic expression to account for the amount lost at daily intervals. For the reactive pool of organic matter, the amount of dissolved organic carbon at each time step, DOC_{rea}^{i}, was given as

$$DOC_{rea}^{i} = DOC_{rea}^{i-1} - DEC_{rea}^{i} \quad (19)$$

Where, $DOC_{rea}^{i-1}$is the mass of reactive organic carbon from the previous time step (mg), DEC_{rea}^{i} is the amount of OC from the reactive pool decomposed to DIC (mg). Processes occurring within the flask are modelled based on a mass balance approach that utilizes first order kinetic expressions. All kinetic subroutines are coupled to a modified Arrhenius expression for temperature.

The first-order subroutine is defined as a process that depends on the amount of a given constituent, and is represented as a rate constant (k) according to first-order kinetics:

$$\frac{dM}{dt} = -kM$$

Integration of this equation yields: $M_i = M_{i-1}e^{-k\Delta t} \times \theta^{(T_{(i)}-T_{ref})}$

With M_i representing the amount processed (mg), M_{i-1} the amount processing in the previous time step, k is the first-order rate constant (day^{-1}), θ is the temperature coefficient (equal to 1.08) T in reference to measured temperature (°C), and Δt as change in time (days).

$$DOC_{rea}^{i} = DOC_{rea}^{i-1} \exp(-k_{rea}\Delta t) \times \theta_{dec}^{(T_{(i)}-T_{ref})} \quad (20)$$

$$DOC_{rea}^{i} - DOC_{rea}^{i-1} = DEC_{rea}^{i} \quad (21)$$

The mineralization of organic nitrogen is coupled to the decomposition of organic carbon via the C/N ratio of each source pool.

$$MIN_{rea}^{i} = \frac{DEC_{rea}^{i}}{C/N_{rea}} \quad (22)$$

The amount of N within a pool at time step i is calculated using the relationship between the C/N ratio of each pool.

$$DON_{rea}^{i} = \frac{DOC_{rea}^{i}}{C/N_{rea}} \tag{23}$$

The more resistant pool of DOM follows the same set of equations.

$$DOC_{res}^{i} = DOC_{res}^{i-1} - DEC_{res}^{i}$$

$$DON_{res}^{i} = DON_{res}^{i-1} - MIN_{res}^{i}$$

$$DOC_{res}^{i} = DOC_{res}^{i-1} \exp(-k_{res}\Delta t) \times \theta_{dec}^{(T_{(i)}-T_{ref})}$$

$$DOC_{res}^{i} - DOC_{res}^{i-1} = DEC_{res}^{i}$$

$$MIN_{res}^{i} = \frac{DEC_{res}^{i}}{C/N_{res}}$$

$$DON_{res}^{i} = \frac{DOC_{res}^{i}}{C/N_{res}}$$

The total amount of dissolved organic carbon and nitrogen is the summation of each pool at the desired time step (i).

$$DOC_{T}^{i} = DOC_{rea}^{i} + DOC_{res}^{i} \tag{24}$$

$$DON_{T}^{i} = DON_{rea}^{i} + DON_{res}^{i}$$

The current percent contribution from each pool and the new C/N ratio of the sediment is calculated as follows.

$$P_{rea}^{i} = \frac{DOC_{rea}^{i}}{DOC_{T}^{i}}$$

$$P_{res}^{i} = \frac{DOC_{res}^{i}}{DOC_{T}^{i}}$$

$$DOM\frac{C}{N}^{i} = \left(P_{rea}^{i} * C/N_{rea}\right) + \left(P_{res}^{i} * C/N_{res}\right) \quad (25)$$

Redox Processes and mass balance

We recognize that many biochemical or redox processes could be impacting the carbon and nitrogen species within incubations, however, only processes deemed relevant and occurring are considered within modelling equations. These processes include carbon oxidation, mineralization, nitrification, and CO_2 evasion. Other processes, such as assimilation and denitrification, are omitted from modelling and further discussed below.

Assimilation is the biotic fixation of NH_3 and NO_3 into microbial biomass. The assimilation process is neglected in the N mass balance model for the dark incubations because primary production dominates in low-order streams as compared to heterotrophic fixation (Birgand, 2007; Kendall, 2007; Ford and Fox, 2014).

Denitrification is the biotic reduction of NO_3 to N_2, as well as intermediate nitrogenous gases such as nitric oxide (NO) and nitrous oxide (N_2O). The nitrate removal process is performed by facultative anaerobic organisms (Birgand, 2007), and is therefore neglected in modeling due to the presence of oxygen within incubated systems.

Nitrification is impacted by amount of NH_3 in the water and temperature and we represent this relationship with first-order kinetics coupled to a modified Arrhenius expression for temperature, as others have (Bowie et al., 1985; Ryzhakov et al., 2010; Husic et al., 2020), as:

$$NH4^{i} = NH4^{i-1/2}\exp(-k_{nit}\Delta t) \times \theta_{nit}^{(T_{(i)}-T_{ref})} \quad (26)$$

Where $NH4^{i}$ is the N nitrified at time step (i) (mg), $NH4^{i-1/2}$ is the amount ammonium from the multiple sources of mineralization during the time step (mg), k_{nit} is the first order rate constant for nitrification (day^{-1}), and θ_{nit} is the nitrification temperature coefficient (equal to 1.08).

$$NH4^{i-\frac{1}{2}} = NH4^{i-1} + MIN_{alg}^{i} + MIN_{soil}^{i} + MIN_{rea}^{i} + MIN_{res}^{i} \quad (27)$$

$$NH4^{i} = NH4^{i-1/2}\exp(-k_{nit}\Delta t) \times \theta_{nit}^{(T_{(i)}-T_{ref})} \quad (28)$$

$$NH4^{i} - NH4^{i-\frac{1}{2}} = NIT^{i} \quad (29)$$

Only relevant transformations are applied to each N pool.

$$SN^{i} = SN^{i-1} - MIN_{alg}^{i} - MIN_{soil}^{i} \quad (30)$$

$$DON^{i} = DON^{i-1} - MIN_{rea}^{i} - MIN_{res}^{i} \quad (31)$$

$$NO3^{i} = NO3^{i-1} + NIT^{i} \quad (32)$$

The only hydrological process considered in numerical modeling equations is the evasion of CO_2 from the water to the atmosphere, based on the assumption the flasks are super-saturated in CO_2 via DIC release during mineralization and respiration (Ford et al., 2015). This term only applies for open flaks that are sensitive to carbon equilibrium kinetics.

$$DIC^{i} = DIC^{i-1/2}\exp(-k_{eva}\Delta t) \times \theta_{eva}^{(T_{(i)}-T_{ref})} \quad (33)$$

Where DIC^i is the CO_2 evaded at time step (i) (mg), $DIC^{i-1/2}$ is the amount DIC from the multiple sources of decomposition during the time step (mg), k_{eva} is the first order rate constant for evasion (day^{-1}), and θ_{eva} is the evasion temperature coefficient (equal to 1.08).

Only relevant transformations are applied to each C pool.

$$SOC^i = SOC^{i-1} - DEC^i_{alg} - DEC^i_{soil} \quad (34)$$

$$DOC^i = DOC^{i-1} - DEC^i_{rea} - DEC^i_{res} \quad (35)$$

$$DIC^{i-\frac{1}{2}} = DIC^{i-1} + DEC^i_{alg} + DEC^i_{soil} + DEC^i_{rea} + DEC^i_{res} \quad (36)$$

Isotope Modelling

Delta Notation: $\delta(‰) = \left(\frac{R_{smpl} - R_{std}}{R_{std}}\right) x1000$ $\qquad R = \frac{[^mX]}{[^nX]}$

Fractionation Factor: α_{A-B} is the partitioning of stable isotopes between two substances A and B

$$\alpha_{A-B} = \frac{R_A}{R_B} = \frac{\delta^m_A + 1000}{\delta^m_B + 1000} = f^{(\alpha-1)} = \left(\frac{X}{X_0}\right)^{(\alpha-1)}$$

f is the fraction remaining after process occurs

Enrichment Factor: $\varepsilon(‰) = (\alpha - 1)x1000$

The Rayleigh equation is used to describe isotopic fractionation processes under the following assumptions: (1) in a mixed system, material is continuously removed that contains molecules of at least two isotopic species (e.g., nitrate with ^{15}N and ^{14}N), (2) the

fractionation associated with the removal process at any instant may be described by the fractionation factor and the enrichment factor, and (3) the fractionation factor and enrichment factor remain constant during the process (Kendall and Caldwell, 1998). The Rayleigh equation may be described as:

$$\delta^M X_B = \delta^M X_A - \varepsilon_{rxn} \ln(f_{B-A})$$

Where A and B are the two substances (or product and substrate), X is the isotope, n is the total number of fractionation processes, and f is the fraction remaining after the process occurs.

Rayleigh Fractionation Isotope Model

The fractionation processes can be represented by discretizing the system temporally, allowing for the Rayleigh equation formulation to be a substances isotopic value at timestep *i* equivalent to the sum of relevant enrichment processes and the substances isotopic value at the previous timestep (*i-1*).

$$\delta^M X_i = \delta^M X_{i-1} - \varepsilon \ln(f)$$

Nitrogen and Carbon pools and processes included with each model equation is defined in the elemental mass balance formulation.

Rayleigh Model - Nitrogen

Isotope modelling for this study is done by coupling the Rayleigh fractionation model with an isotope mixing model to quantify source contributions to a mixture.

$$SN_{alg}^{i-1} = SN_{alg}^{i} + MIN_{alg}^{i}$$

$$\delta^{15}N_{alg}^{SN\,i} = \delta^{15}N_{alg}^{SN\,i-1} - \varepsilon_{alg\,min}^{SN}\, x\, \ln\left(\frac{SN_{alg}^{i}}{SN_{alg}^{i-1}}\right) \quad (37)$$

Where $\delta^{15}N_{alg}^{SN\,i}$ is the isotopic signature of algal nitrogen at time step i, $\delta^{15}N_{alg}^{SN\,i-1}$ is the isotopic signature of algal nitrogen at the previous time step, and $\varepsilon_{alg\,min}^{SN}$ is the isotope enrichment factor for the mineralization of the algal nitrogen.

$$SN_{soil}^{i-1} = SN_{soil}^{i} + MIN_{soil}^{i}$$

$$\delta^{15}N_{soil}^{SN\,i} = \delta^{15}N_{soil}^{SN\,i-1} - \varepsilon_{soil\,min}^{SN}\, x\, \ln\left(\frac{SN_{soil}^{i}}{SN_{soil}^{i-1}}\right) \quad (38)$$

Where $\delta^{15}N_{soil}^{SN\,i}$ is the isotopic signature of soil nitrogen at time step i, $\delta^{15}N_{soil}^{SN\,i-1}$ is the isotopic signature of soil nitrogen at the previous time step, and $\varepsilon_{soil\,min}^{SN}$ is the isotope enrichment factor for the mineralization of the soil nitrogen.

The isotopic signature of the sediment nitrogen is estimated by taking the product of each sources isotope signature and its relative contribution to the sediment nitrogen pool.

$$\delta^{15}N_{T}^{SN\,i} = \delta^{15}N_{alg}^{SN\,i}\left(P_{alg_i}\right) + \delta^{15}N_{soil}^{SN\,i}\left(P_{soil_i}\right) \quad (39)$$

A similar approach for the mineralization of dissolved organic nitrogen is used by coupling Rayleigh fractionation equations with an isotope mixing model.

$$DON_{rea}^{i-1} = DON_{rea}^{i} + MIN_{rea}^{i}$$

$$\delta^{15}N_{rea}^{DOM\,i} = \delta^{15}N_{rea}^{DOM\,i-1} - \varepsilon_{rea\,min}^{DOM}\, x\, \ln\left(\frac{DON_{rea}^{i}}{DON_{rea}^{i-1}}\right) \quad (40)$$

Where $\delta^{15}N_{rea}^{DOM^{i}}$ is the isotopic signature of reactive nitrogen at time step i, $\delta^{15}N_{rea}^{DOM^{i-1}}$ is the isotopic signature of reactive nitrogen at the previous time step, and $\varepsilon_{rea\,min}^{DOM}$ is the isotope enrichment factor for the mineralization of the reactive nitrogen.

$$DON_{res}^{i-1} = DON_{res}^{i} + MIN_{res}^{i}$$

$$\delta^{15}N_{res}^{DOM^{i}} = \delta^{15}N_{res}^{DOM^{i-1}} - \varepsilon_{res\,min}^{DOM}\ x\ \ln\left(\frac{DON_{res}^{i}}{DON_{res}^{i-1}}\right) \quad (41)$$

Where $\delta^{15}N_{res}^{DOM^{i}}$ is the isotopic signature of resistant nitrogen at time step i, $\delta^{15}N_{res}^{DOM^{i-1}}$ is the isotopic signature of resistant nitrogen at the previous time step, and $\varepsilon_{res\,min}^{DOM}$ is the isotope enrichment factor for the mineralization of the resistant nitrogen.

The isotopic signature of the dissolved organic nitrogen is estimated by taking the product of each sources isotope signature and its relative contribution to the DOM nitrogen pool.

$$\delta^{15}N_{T}^{DOM^{i}} = \delta^{15}N_{rea}^{DOM^{i}}\left(P_{rea_i}\right) + \delta^{15}N_{res}^{DOM^{i}}\left(P_{res_i}\right) \quad (42)$$

An isotope unmixing model was then used to estimate each pools isotopic contribution to the sink term for each process, in this case the isotopic signature of ammonium coming from each pool of sediment.

Sediment N Isotope Unmixing Model

$$SN_{alg}^{i-1}\delta^{15}N_{alg}^{SN\,i-1} = SN_{alg}^{i}\delta^{15}N_{alg}^{SN\,i} + MIN_{alg}^{i}\delta^{15}N_{alg}^{NH4^{i}}$$

$$\delta^{15}N_{alg}^{NH4^{i}} = \frac{SN_{alg}^{i-1}\delta^{15}N_{alg}^{SN\,i-1} - SN_{alg}^{i}\delta^{15}N_{alg}^{SN\,i}}{MIN_{alg}^{i}} \quad (43)$$

$$SN_{soil}^{i-1}\delta^{15}N_{soil}^{SN\,i-1} = SN_{soil}^{i}\delta^{15}N_{soil}^{SN\,i} + MIN_{soil}^{i}\delta^{15}N_{soil}^{NH4^{i}}$$

$$\delta^{15}N_{soil}^{NH4^i} = \frac{SN_{soil}^{i-1}\delta^{15}N_{soil}^{SN^{i-1}} - SN_{soil}^{i}\delta^{15}N_{soil}^{SN^{i}}}{MIN_{soil}^{i}} \quad (44)$$

DOM N Isotope Unmixing Model

$$DON_{rea}^{i-1}\delta^{15}N_{rea}^{DON^{i-1}} = DON_{rea}^{i}\delta^{15}N_{rea}^{DON^{i}} + MIN_{rea}^{i}\delta^{15}N_{rea}^{NH4^i}$$

$$\delta^{15}N_{rea}^{NH4^i} = \frac{DON_{rea}^{i-1}\delta^{15}N_{rea}^{DON^{i-1}} - DON_{rea}^{i}\delta^{15}N_{rea}^{DON^{i}}}{MIN_{rea}^{i}} \quad (45)$$

$$DON_{res}^{i-1}\delta^{15}N_{res}^{DON^{i-1}} = DON_{res}^{i}\delta^{15}N_{res}^{DON^{i}} + MIN_{res}^{i}\delta^{15}N_{res}^{NH4^i}$$

$$\delta^{15}N_{res}^{NH4^i} = \frac{DON_{res}^{i-1}\delta^{15}N_{res}^{DON^{i-1}} - DON_{res}^{i}\delta^{15}N_{res}^{DON^{i}}}{MIN_{res}^{i}} \quad (46)$$

The fraction of a substrate mineralized to ammonium is the amount of a pool lost divided by the total N mineralized from all pools.

$$MIN_T^i = MIN_{alg}^i + MIN_{soil}^i + MIN_{rea}^i + MIN_{res}^i \quad (47)$$

$$X_{alg}^i = \frac{MIN_{alg}^i}{MIN_T^i} \qquad X_{soil}^i = \frac{MIN_{soil}^i}{MIN_T^i} \qquad X_{rea}^i = \frac{MIN_{rea}^i}{MIN_T^i} \qquad X_{res}^i = \frac{MIN_{res}^i}{MIN_T^i}$$

$$X_{alg}^i + X_{soil}^i + X_{rea}^i + X_{res}^i = 1 \quad (48)$$

The isotope mixing of substrates mineralized to ammonium is the sum of the pool isotope value multiplied by the fraction of the pool mineralized.

$$\delta^{15}N_{alg}^{NH4^i} = \frac{SN_{alg}^{i-1}\delta^{15}N_{alg}^{SN^{i-1}} - SN_{alg}^{i}\delta^{15}N_{alg}^{SN^{i}}}{MIN_{alg}^{i}}$$

$$\delta^{15}N_{soil}^{NH4^i} = \frac{SN_{soil}^{i-1}\delta^{15}N_{soil}^{SN^{i-1}} - SN_{soil}^{i}\delta^{15}N_{soil}^{SN^{i}}}{MIN_{soil}^{i}}$$

$$\delta^{15}N_{rea}^{NH4^i} = \frac{DON_{rea}^{i-1}\delta^{15}N_{rea}^{DON^{i-1}} - DON_{rea}^{i}\delta^{15}N_{rea}^{DON^i}}{MIN_{rea}^{i}}$$

$$\delta^{15}N_{res}^{NH4^i} = \frac{DON_{res}^{i-1}\delta^{15}N_{res}^{DON^{i-1}} - DON_{res}^{i}\delta^{15}N_{res}^{DON^i}}{MIN_{res}^{i}}$$

$$\delta^{15}N_{M}^{NH4^i} = \left[(X_{alg}^{i})\delta^{15}N_{alg}^{NH4^i} + (X_{soil}^{i})\delta^{15}N_{soil}^{NH4^i} + (X_{rea}^{i})\delta^{15}N_{rea}^{NH4^i} + (X_{res}^{i})\delta^{15}N_{res}^{NH4^i}\right] \quad (49)$$

The ammonium isotope value is the mixing of the mineralized organic matter pools and the ammonium isotope value from the previous time step minus the amount nitrified.

$$Y_{M}^{i} = \frac{MIN_{T}^{i}}{MIN_{T}^{i} + NH_{4}^{i-1}} \quad (50)$$

$$Y_{NH4}^{i-1} = \frac{NH_{4}^{i-1}}{MIN_{T}^{i} + NH_{4}^{i-1}} \quad (51)$$

$$\delta^{15}N_{NH4}{}^{i-1/2} = Y_{M}^{i}\delta^{15}N_{M}^{NH4^i} + Y_{nh4}^{i-1}\delta^{15}N_{NH4}{}^{i-1} \quad (52)$$

$$\delta^{15}N_{NH4}{}^{i} = \delta^{15}N_{NH4}{}^{i-1/2} - \varepsilon_{NIT}\ln\left(\frac{NH_{4}^{i}}{NH_{4}^{i-1/2}}\right) \quad (53)$$

$$\delta^{15}N_{NO3}{}^{i} = \varepsilon_{NIT}\ x\ \ln\left(\frac{NH_{4}^{i}}{NH_{4}^{i-1/2}}\right) + \delta^{15}N_{NO3}{}^{i-1} \quad (54)$$

Rayleigh Model - Carbon

Isotope modelling for this study is done by coupling the Rayleigh fractionation model with an isotope mixing model to quantify source contributions to a mixture.

$$SOC_{alg}^{i-1} = SOC_{alg}^{i} + DEC_{alg}^{i}$$

$$\delta^{13}C_{alg}^{SOC^i} = \delta^{13}C_{alg}^{SOC^{i-1}} - \varepsilon_{alg\,dec}^{SOC}\, x\, \ln\left(\frac{SOC_{alg}^{i}}{SOC_{alg}^{i-1}}\right) \quad (55)$$

Where $\delta^{13}C_{alg}^{SOC^i}$ is the isotopic signature of algal carbon at time step i, $\delta^{13}C_{alg}^{SOC^{i-1}}$ is the isotopic signature of algal carbon at the previous time step, and $\varepsilon_{alg\,dec}^{SOC}$ is the isotope enrichment factor for the decomposition of the algal carbon.

$$SOC_{soil}^{i-1} = SOC_{soil}^{i} + DEC_{soil}^{i}$$

$$\delta^{13}C_{soil}^{SOC^i} = \delta^{13}C_{soil}^{SOC^{i-1}} - \varepsilon_{soil\,dec}^{SOC}\, x\, \ln\left(\frac{SOC_{soil}^{i}}{SOC_{soil}^{i-1}}\right) \quad (56)$$

Where $\delta^{13}C_{soil}^{SOC^i}$ is the isotopic signature of soil carbon at time step i, $\delta^{13}C_{soil}^{SOC^{i-1}}$ is the isotopic signature of soil carbon at the previous time step, and $\varepsilon_{soil\,dec}^{SOC}$ is the isotope enrichment factor for the decomposition of the soil carbon.

The isotopic signature of the sediment carbon is estimated by taking the product of each sources isotope signature and its relative contribution to the sediment carbon pool.

$$\delta^{13}C_{T}^{SOC^i} = \delta^{13}C_{alg}^{SOC^i}\left(P_{alg_i}\right) + \delta^{13}C_{soil}^{SOC^i}\left(P_{soil_i}\right) \quad (57)$$

A similar approach for the decomposition of dissolved organic carbon is used by coupling Rayleigh fractionation equations with an isotope mixing model.

$$DOC_{rea}^{i-1} = DOC_{rea}^{i} + DEC_{rea}^{i}$$

$$\delta^{13}C_{rea}^{DOM^i} = \delta^{13}C_{rea}^{DOM^{i-1}} - \varepsilon_{rea\,dec}^{DOM}\, x\, \ln\left(\frac{DOC_{rea}^{i}}{DOC_{rea}^{i-1}}\right) \quad (58)$$

Where $\delta^{13}C_{rea}^{DOM^i}$ is the isotopic signature of reactive carbon at time step i, $\delta^{13}C_{rea}^{DOM^{i-1}}$ is the isotopic signature of reactive carbon at the previous time step, and $\varepsilon_{rea\,dec}^{DOM}$ is the isotope enrichment factor for the decomposition of the reactive carbon.

$$DOC_{res}^{i-1} = DOC_{res}^{i} + DEC_{res}^{i}$$

$$\delta^{13}C_{res}^{DOM^i} = \delta^{13}C_{res}^{DOM^{i-1}} - \varepsilon_{res\,dec}^{DOM}\, x\, \ln\left(\frac{DOC_{res}^{i}}{DOC_{res}^{i-1}}\right) \quad (59)$$

Where $\delta^{13}C_{res}^{DOM^i}$ is the isotopic signature of resistant carbon at time step i, $\delta^{13}C_{res}^{DOM^{i-1}}$ is the isotopic signature of resistant carbon at the previous time step, and $\varepsilon_{res\,dec}^{DOM}$ is the isotope enrichment factor for the decomposition of resistant carbon.

The isotopic signature of the dissolved organic carbon is estimated by taking the product of each sources isotope signature and its relative contribution to the DOM carbon pool.

$$\delta^{13}C_{T}^{DOM^i} = \delta^{13}C_{rea}^{DOM^i}\left(P_{rea_i}\right) + \delta^{13}C_{res}^{DOM^i}\left(P_{res_i}\right) \quad (60)$$

An isotope unmixing model was then used to estimate each pools isotopic contribution to the sink term for each process, in this case the isotopic signature of dissolved inorganic carbon coming from each pool of sediment.

Sediment C Isotope Unmixing Model

$$SOC_{alg}^{i-1}\delta^{13}C_{alg}^{SOC^{i-1}} = SOC_{alg}^{i}\delta^{13}C_{alg}^{SOC^i} + DEC_{alg}^{i}\delta^{13}C_{alg}^{DIC^i}$$

$$\delta^{13}C_{alg}^{DIC^i} = \frac{SOC_{alg}^{i-1}\delta^{13}C_{alg}^{SOC^{i-1}} - SOC_{alg}^{i}\delta^{13}C_{alg}^{SOC^i}}{DEC_{alg}^{i}} \quad (61)$$

$$SOC_{soil}^{i-1}\delta^{13}C_{soil}^{SOC^{i-1}} = SOC_{soil}^{i}\delta^{13}C_{soil}^{SOC^i} + DEC_{soil}^{i}\delta^{13}C_{soil}^{DIC^i}$$

$$\delta^{13}C_{soil}^{DIC^i} = \frac{SOC_{soil}^{i-1}\delta^{13}C_{soil}^{SOC^{i-1}} - SOC_{soil}^{i}\delta^{13}C_{soil}^{SOC^i}}{DEC_{soil}^{i}} \quad (62)$$

DOM C Isotope Unmixing Model

$$DOC_{rea}^{i-1}\delta^{13}C_{rea}^{DOC^{i-1}} = DOC_{rea}^{i}\delta^{13}C_{rea}^{DOC^i} + DEC_{rea}^{i}\delta^{13}C_{rea}^{DIC^i}$$

$$\delta^{13}C_{rea}^{DIC^i} = \frac{DOC_{rea}^{i-1}\delta^{13}C_{rea}^{DOC^{i-1}} - DOC_{rea}^{i}\delta^{13}C_{rea}^{DOC^i}}{DEC_{rea}^{i}} \quad (63)$$

$$DOC_{res}^{i-1}\delta^{13}C_{res}^{DOC^{i-1}} = DOC_{res}^{i}\delta^{13}C_{res}^{DOC^i} + DEC_{res}^{i}\delta^{13}C_{res}^{DIC^i}$$

$$\delta^{13}C_{res}^{DIC^i} = \frac{DOC_{res}^{i-1}\delta^{13}C_{res}^{DOC^{i-1}} - DOC_{res}^{i}\delta^{13}C_{res}^{DOC^i}}{DEC_{res}^{i}} \quad (64)$$

The fraction of a substrate decomposed to DIC is the amount of a pool lost divided by the total C decomposed from all pools.

$$DEC_T^i = DEC_{alg}^i + DEC_{soil}^i + DEC_{rea}^i + DEC_{res}^i \quad (65)$$

$$X_{alg}^i = \frac{DEC_{alg}^i}{DEC_T^i} \qquad X_{soil}^i = \frac{DEC_{soil}^i}{DEC_T^i} \qquad X_{rea}^i = \frac{DEC_{rea}^i}{DEC_T^i} \qquad X_{res}^i = \frac{DEC_{res}^i}{DEC_T^i}$$

$$X_{alg}^i + X_{soil}^i + X_{rea}^i + X_{res}^i = 1 \quad (66)$$

The isotope mixing of substrates decomposed to DIC is the sum of the pool isotope value multiplied by the fraction of the pool decomposed.

$$\delta^{13}C_{alg}^{DIC^i} = \frac{SOC_{alg}^{i-1}\delta^{13}C_{alg}^{SOC^{i-1}} - SOC_{alg}^{i}\delta^{13}C_{alg}^{SOC^i}}{DEC_{alg}^{i}}$$

$$\delta^{13}C_{soil}^{DIC^i} = \frac{SOC_{soil}^{i-1}\delta^{13}C_{soil}^{SOC^{i-1}} - SOC_{soil}^{i}\delta^{13}C_{soil}^{SOC^i}}{DEC_{soil}^{i}}$$

$$\delta^{13}C_{rea}^{DIC^i} = \frac{DOC_{rea}^{i-1}\delta^{13}C_{rea}^{DOC^{i-1}} - DOC_{rea}^{i}\delta^{13}C_{rea}^{DOC^i}}{DEC_{rea}^{i}}$$

$$\delta^{13}C_{res}^{DIC^i} = \frac{DOC_{res}^{i-1}\delta^{13}C_{res}^{DOC^{i-1}} - DOC_{res}^{i}\delta^{13}C_{res}^{DOC^i}}{DEC_{res}^{i}}$$

$$\delta^{13}C_{M}^{DIC^i} = \left[(X_{alg}^{i})\delta^{13}C_{alg}^{DIC^i} + (X_{soil}^{i})\delta^{13}C_{soil}^{DIC^i} + (X_{rea}^{i})\delta^{13}C_{rea}^{DIC^i} + (X_{res}^{i})\delta^{13}C_{res}^{DIC^i}\right] \quad (67)$$

The DIC isotope value is the mixing of the decomposed OM pools minus the amount evaded plus the DIC isotope value from the previous time step.

$$Y_{M}^{i} = \frac{DEC_{T}^{i}}{DEC_{T}^{i} + DIC^{i-1}} \quad (68)$$

$$Y_{DIC}^{i-1} = \frac{DIC^{i-1}}{DEC_{T}^{i} + DIC^{i-1}} \quad (69)$$

$$\delta^{13}C_{DIC}{}^{i-1/2} = Y_{M}^{i}\delta^{13}C_{M}^{DIC^i} + Y_{DIC}^{i-1}\delta^{13}C_{DIC}{}^{i-1} \quad (70)$$

$$\delta^{13}C_{DIC}{}^{i} = \delta^{13}C_{DIC}{}^{i-1/2} - \varepsilon_{EVA}\ln\left(\frac{DIC^{i}}{DIC^{i-\frac{1}{2}}}\right) \quad (71)$$

3.5 Analyze fluvial sediment during high flow events

Collect and analyze fluvial sediment provenance and conservativeness during high flow and extreme flow hydrologic events using field measurements of C and N elemental and isotope values:

For this study, elemental and isotopic analysis were performed on sediment trap samples (following Phillips et al., 2000, see Figure 7) collected within South Elkhorn Creek from 2014-2017. The trap sampler is composed of PVC pipe, which is cleaned and rinsed with DI water after each use. Sediment samples were collected at Ramsey's located at the

mid-point of the watershed (~30 km^2) and draining the upper catchment; and at Gage located at the watershed outlet (62 km^2) and draining both the upper and lower catchment. Analysis of sediment organic matter is applied to both the carbon (SOC and $\delta^{13}C$) and nitrogen (SN and $\delta^{15}N$) composition of the less than 53-μm fraction. The organic signature serves as a potential means for partitioning contributions of upland soils versus autochthonous streambed sediments. Transported sediments are used in this study as an integrated signal that account for spatial variability, rather than collecting a sediment source from a single point or sets of points in the watershed.

Sediment samples were brought back to lab and processed for elemental analysis through centrifugation, freezing, freeze drying, consolidating and weighing, wet sieving and elemental analyses. Sediment trap samples are collected in 5 gallon buckets, then stored in the refrigerator at 4°C and settled for 48 hours. Once settled, water is siphoned off and the remaining sediment-water mixture is poured into 750 mL bottles for centrifugation. Samples were centrifuged at 3000 rpm for 10 minutes. The 750 mL bottles are decanted and centrifuged such that only a thin layer of water remains without disturbing sediment. The bottles were then placed in a freezer overnight or until the sample was completely frozen. Frozen sediment samples are lyophilized using a FreeZone® Freeze Dry System where pressure and temperature were reduced down to 0.5 mbar and -50°C, respectively. The freeze drying process is about 2 to 3 days, and results in a dried, stead-state sediment sample.

Depending on the total weight of the sample, a subsamples of the entire sample, 0.5 g, 1.0 g, or 2.0 g was separated from the bulk sample. Subsamples of the steady-state sediment were subjected to a wet sieving procedure, using a #270 mesh to separate fine

(<53 μm) and coarse particulates. Rewetting the sediment required another round of lab processing, including centrifuging, decanting, and freeze drying for the fine sediment sample. Once completely dry, the mass of the remaining fine fraction of the sediment was measured and recorded.

Fine sediment samples were then ground to a fine powder using a Sigma-Aldrich Wig-L-Bug® grinder to be easily combustible during elemental analysis. Ground samples were weighed into tin capsules and acidified with 0.5 M hydrochloric acid to remove any inorganic carbonate phases in the sample (Dabundo and Munizzi, 2018). Samples were analyzed in a Costech ® 4010 elemental analyzer coupled to a Finnigan Isotope Ratio Mass Spectrometer which estimated both percent carbon and nitrogen as well as $\delta^{13}C$ and $\delta^{15}N$ of the sediment.

A high flow period is defined by a peak discharge greater than 2.8 m^3/s (~100 ft^3/s) measured at USGS gage for South Elkhorn Creek at Fort Spring, KY. The USGS gage is located about 100 feet downstream from the outlet of the watershed, the sampling site labeled Gage. Any trap that was in-stream during a peak flow greater than 2.8 m^3/s was defined as a high flow, or extreme, event sample.

3.6 Analyze fluvial sediment during low flow events

Collect and analyze changes occurring to fluvial sediment organic matter during temporarily storage as well as tracer (non)conservativeness using field measurements of C and N elemental and isotope values:

Sediment samples collected for this section of the study were subjected to the field and laboratory methodology described in section 3.5. All sediment traps that were in-stream during a peak flow below 2.8 m^3/s were classified as low flow samples. It is expected that

the creek is relatively stagnant during these periods, allowing for temporary storage of sediment within the streambed.

3.7 Compare fluvial sediment with laboratory incubations

Compare field-based fluvial sediment agreement with laboratory incubation results by assessing seasonal and flow regime dependence of field measurements of C and N elemental and isotope values:

Sediment samples collected for this section of the study were subjected to the field and laboratory methodology described in section 3.5. All sediment data was analyzed by both seasonality and defined flow regime. Data collected during low flow periods of summer months were considered to be in best agreement with results of laboratory incubations.

Table 3-1: Summary of variables measured as part of this thesis

Name	**Description**	**No. of Samples**
DO	A measure of the amount of oxygen dissolved in the water	N/A
pH	A measure of the acidic/basic level of water	N/A
Temp	A measure of the temperature in the controlled-environment	N/A
NO_3^-	A measure of Nitrate concentrations in the water	92
NH_4^+	A measure of Ammonium concentrations in the water	92
TKN	A measure of Total Kjeldahl Nitrogen concentrations in water	92
DOC	A measure of Dissolved Organic Carbon concentrations in the water	92
DIC	A measure of Dissolved Inorganic Carbon concentrations in the water	92
$\delta^{13}C_{DIC}$	A measure of the Carbon isotope ratio in a **water** sample	92
$\delta^{15}N_{NO3}$	A measure of the Nitrogen isotope ratio in a **nitrate** sample	92
$\delta^{18}O_{NO3}$	A measure of the Oxygen isotope ratio in a **nitrate** sample	92
SOC	A measure of the Total Organic Carbon within a sediment sample	86
SN	A measure of total NO_3-N, NO_2-N, and organic N in a sediment sample	86
$\delta^{13}C_{Sed}$	A measure of the Carbon isotope ratio in a **sediment** sample	86
$\delta^{15}N_{Sed}$	A measure of the Nitrogen isotope ratio in a **sediment** sample	86

Table 3-2: Mean sediment data of pooled archived samples used in incubation experiments

Sediment Classification	Mean (SD) $\delta^{13}C$ (‰)	Mean (SD) $\delta^{15}N$ (‰)	Mean (SD) SOC (%)	Mean (SD) SN (%)	Mean (SD) C/N Ratio
Upland (n=5)	-25.98 (0.28)	5.49 (0.23)	2.08 (0.26)	0.23 (0.03)	10.6 (0.37)
Instream (n=12)	-27.10 (0.16)	3.98 (0.67)	3.63 (0.39)	0.36 (0.04)	11.8 (0.67)

Table 3-3: Archived sediment trap samples representative of upland source contributions

Location	Date	Label	$\delta^{13}C$ (‰)	SOC (%)	$\delta^{15}N$ (‰)	SN (%)	C/N
Gage	9/29/2006	SE 17A	-25.679	1.714	5.815	0.198	10.086
Gage	10/5/2006	SE 18A	-26.096	2.302	5.748	0.242	11.087
Gage	10/25/2006	SE 21A	-26.036	2.309	5.330	0.256	10.508
Gage	11/2/2006	SE 22A	-25.667	1.830	5.521	0.195	10.945
Gage	11/16/2006	SE 24A	-26.405	2.255	5.043	0.254	10.355
Total		**Mean**	**-25.977**	**2.082**	**5.491**	**0.229**	**10.596**
		Std. Dev	0.278	0.257	0.282	0.027	0.371

Table 3-4: Archived sediment trap samples representative of instream source contributions

Location	Date	Label	$\delta^{13}C$ (‰)	SOC (%)	$\delta^{15}N$ (‰)	SN (%)	C/N
Ramsey	7/3/2008	F_R02	-27.130	3.806	2.907	0.356	12.455
Ramsey	7/10/2008	F_R03	-27.001	3.588	3.300	0.332	12.603
Ramsey	7/16/2008	F_R04	-26.746	3.011	5.029	0.286	12.274
Gage	7/16/2008	F_G04	-26.927	2.975	3.127	0.327	10.603
Ramsey	7/29/2008	F_R05	-27.006	3.916	3.354	0.383	11.936
Ramsey	9/4/2008	F_R08	-27.085	3.74	4.409	0.35	12.466
Gage	9/11/2008	F_G09	-27.082	3.879	4.689	0.384	11.780
Gage	9/19/2008	F_G10	-27.149	3.419	4.348	0.358	11.136
Gage	12/5/2008	F_G16	-27.398	3.827	4.435	0.415	10.757
Gage	12/17/2008	F_G17	-27.151	3.175	4.388	0.329	11.262
Ramsey	12/17/2008	F_R17	-27.189	4.295	4.241	0.412	12.158
Ramsey	1/8/2009	F_R18	-27.280	3.964	3.506	0.374	12.361
Total		**Mean**	**-27.095**	**3.633**	**3.978**	**0.359**	**11.816**
		Std. Dev	0.161	0.392	0.666	0.036	0.674

Table 3-5: Batch study experimental setup and total number of samples sent for analyses

Period	Samples Sent for Analysis											
0 (1) *6-Apr-19*	U-0a	I-0a			OB-0a	AB-0a						
	U-0b	I-0b			OB-0b	AB-0b						
	U-0c	I-0c			OB-0c	AB-0c						
1 (3) *9-Apr-19*	OU-1a	OI-1a	AU-1a	AI-1a								
	OU-1b	OI-1b	AU-1b	AI-1b								
	OU-1a	OI-1c	AU-1c	AI-1c								
2 (7) *13-Apr-19*	OU-2	OI-2	AU-2	AI-2								
3 (14) *20-Apr-19*	OU-3	OI-3	AU-3	AI-3	OB-1	AB-1	OUC-1	AUC-1	OIC-1	AIC-1		
4 (21) *27-Apr-19*	OU-4a	OI-4a	AU-4a	AI-4a								
	OU-4b	OI-4b	AU-4b	AI-4b								
	OU-4c	OI-4c	AU-4c	AI-4c								
5 (28) *4-May-19*	OU-5	OI-5	AU-5	AI-5	OB-2	AB-2	OUC-2	AUC-2	OIC-2	AIC-2		
6 (42) *18-May-19*	OU-6	OI-6	AU-6	AI-6								
7 (56) *1-June-19*	OU-7	OI-7	AU-7	AI-7	OB-3	AB-3	OUC-3a	AUC-3a	OIC-3a	AIC-3a		
							OUC-3b	AUC-3b	OIC-3b	AIC-3b		
							OUC-3c	AUC-3c	OIC-3c	AIC-3c		
8 (70) *15-June-19*	OU-8a	OI-8a	AU-8a	AI-8a								
	OU-8b	OI-8b	AU-8b	AI-8b								
	OU-8c	OI-8c	AU-8c	AI-8c								
9 (140) *24-Aug-19*	OU-9	OI-9	AU-9	AI-9								
Sent for Analysis	Open System		Closed System		Blank System		Sterilized System					
	30		30		12		20					
	Total Samples		92									

Table 3-6: Batch study experimental sampling strategy and analytes

	Solute Samples				Sediment Samples
Analyses:	UASIL		KGS		KSIGL
Period	$\delta^{15}N/\delta^{18}O_{NO3}$	$\delta^{13}C_{DIC}$	DIC, DOC, NO_3, NH_4, TKN		$\delta^{15}N_{sed}$, SN, $\delta^{13}C_{sed}$, SOC
0 (1)	(n=6) (x=6)		(n=6) (x=6)		(n=6) (x=6)
1 (3)	(n=12) (x=12)		(n=12) (x=12)		(n=12) (x=12)
2 (7)	(n=12) (x=4)		(n=12) (x=4)		(n=12) (x=4)
3 (14)	(n=30) (x=10)		(n=30) (x=10)		(n=24) (x=8)
4 (21)	(n=12) (x=12)		(n=12) (x=12)		(n=12) (x=12)
5 (28)	(n=30) (x=10)		(n=30) (x=10)		(n=24) (x=8)
6 (42)	(n=12) (x=4)		(n=12) (x=4)		(n=12) (x=4)
7 (56)	(n=30) (x=18)		(n=30) (x=18)		(n=24) (x=16)
8 (70)	(n=12) (x=12)		(n=12) (x=12)		(n=12) (x=12)
9 (140)	(n=12) (x=4)		(n=12) (x=4)		(n=12) (x=4)
Final Set-Up	n=168	x=92	n=168	x=92	x=86
Initial	6	6	6	6	6
Closed System	54	30	54	30	30
Open System	54	30	54	30	30
Blank Control	18	6	18	6	0
Abiotic Control	36	20	36	20	20
Totals	168	92	168	92	86

n = no. of flasks x = no. of analyses

Period	Open System	Closed System	Abiotic System	Blank System	Triplicate Analyses	Sampling Description
0 (1)				X	X	Triplicate analyses for initial condition
1 (3)	X	X			X	Triplicate analyses with no controls
2 (7)	X	X				Pooled analyses with no controls
3 (14)	X	X	X	X		Pooled analyses with controls
4 (21)	X	X			X	Triplicate analyses with no controls
5 (28)	X	X	X	X		Pooled analyses with controls
6 (42)	X	X				Pooled analyses with no controls
7 (56)	X	X	X	X		Pooled analyses with triplicated control
8 (70)	X	X			X	Triplicate analyses with no controls
9 (140)	X	X				Pooled analyses with no controls

Table 3-7: Inputs and parameters for the elemental and isotopic mass balance models, including parameter description, range of values, units, and reference(s)

Inputs and Parameters	Description	Range of Values	Units	Source for Selected Range
C/N_{alg}	C/N ratio of algal organic matter	6	None	Meyers, 1994; Baird and Middleton, 2004
C/N_{soil}	C/N ratio of soil organic matter	11-13	None	Manzoni and Porporato, 2009
C/N_{rea}	C/N ratio of reactive dissolved organic matter	50	None	Reich and Oleksyn, 2004; Cleveland and Liptzin, 2006; Sinsabaugh, 2009
C/N_{res}	C/N ratio of resistant dissolved organic matter	10	None	Nicolardot et al., 2001
SOC_T	Total amount of sediment organic carbon	3.1 - 5.9	[mg]	Measured
DOC_T	Total amount of dissolved organic carbon	3.5 - 4.4	[mg]	Measured
k_{alg}	First-order algal decomposition rate constant	0.003 - 0.006	[day^{-1}]	Modelled
k_{soil}	First-order soil decomposition rate constant	0.0005 - 0.003	[day^{-1}]	Modelled
k_{rea}	First-order reactive material decomposition rate constant	0.03 - 0.05	[day^{-1}]	Modelled
k_{res}	First-order resistant material decomposition rate constant	0.002	[day^{-1}]	Modelled
k_{nit}	First-order nitrification rate constant	0.2	[day^{-1}]	Modelled
$\varepsilon_{alg\ min}^{SN}$	Algal mineralization enrichment factor	-1 - +1	‰	Modelled
$\varepsilon_{soil\ min}^{SN}$	Soil mineralization enrichment factor	-1 - +1	‰	Modelled
$\varepsilon_{alg\ dec}^{SOC}$	Algal decomposition enrichment factor	-3 - +1	‰	Modelled
$\varepsilon_{soil\ dec}^{SOC}$	Soil decomposition enrichment factor	-3 - +1	‰	Modelled
ε_{nit}	Nitrification enrichment factor	0.05 - 0.2	‰	Modelled

Figure 3-1: South Elkhorn Creek Land Use Map

Study watershed, land use, instream sample site locations (from Fox et al. 2010), and stream location within the Kentucky River Basin, USA. Land use in the upper catchment is primarily urban (60% urban, 40% agricultural). Land use in the lower catchment is primarily agricultural (72% agricultural, 28% urban).

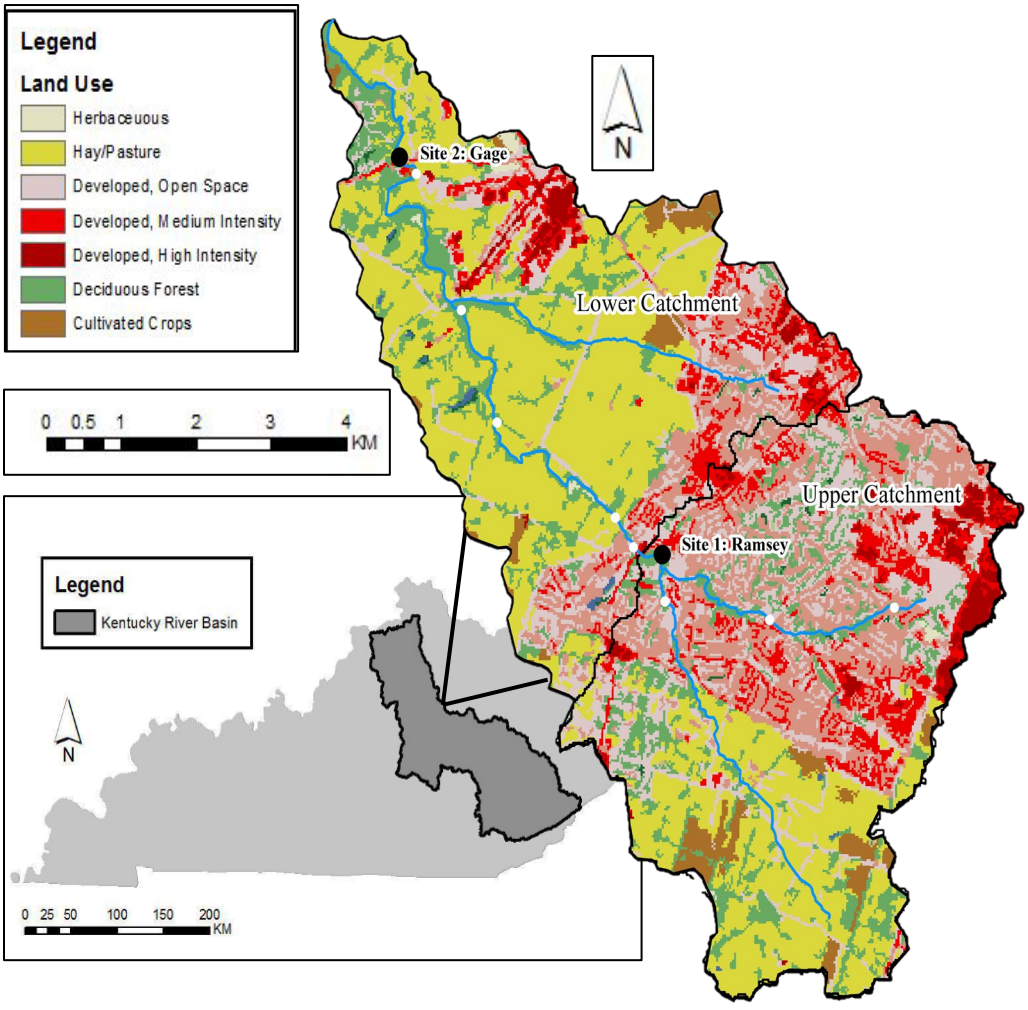


Figure 3-2: Extreme Flow Event Hydrograph

South Elkhorn Creek at Fort Spring, KY (USGS 03289000) spanning fall 2006 to winter 2007, including an extreme flow event (Q_{peak} = 5120 ft^3/s) on September 23, 2006. The $\delta^{13}C$ value of sediments collected at the outlet (Gage) of the watershed reflect surface and subsurface soil samples from hay agriculture known to dominate the basin.

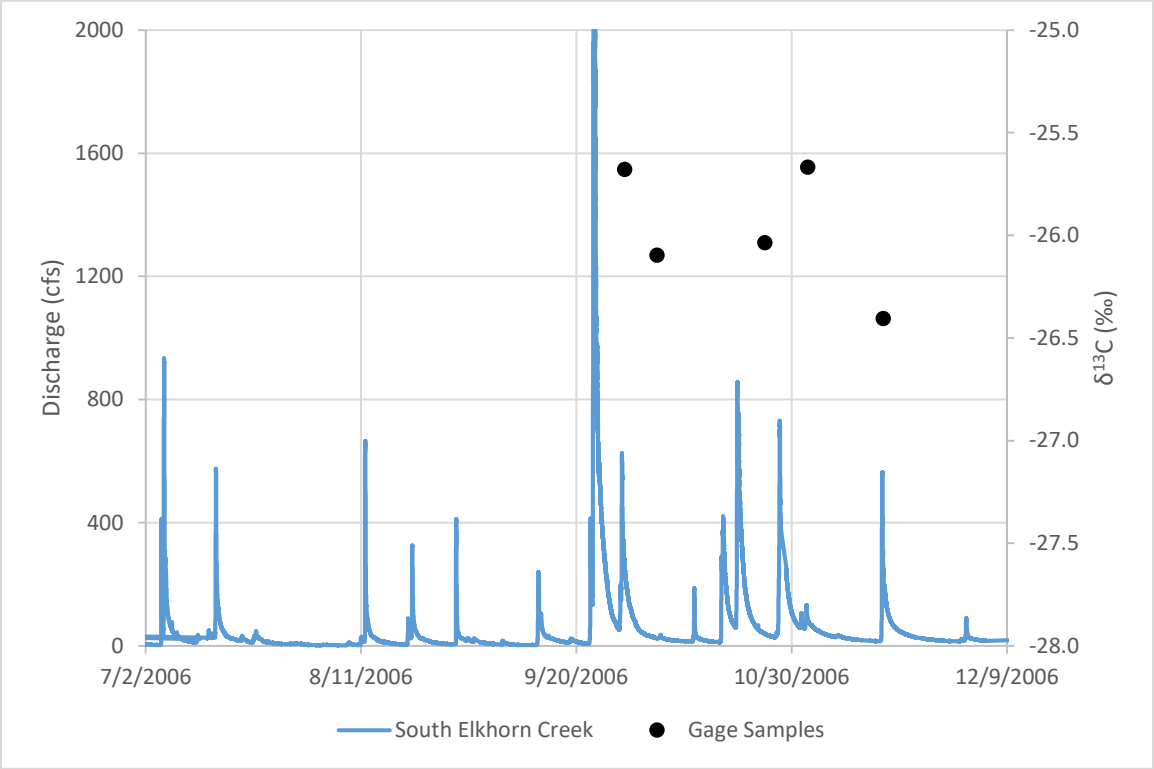


Figure 3-3: Low Flow Event Hydrograph

South Elkhorn Creek at Fort Spring, KY (USGS 03289000) during an extended low to moderate flow event spanning summer 2008 to winter 2009. The $\delta^{13}C$ value of sediments collected at the midpoint (Ramsey) and outlet (Gage) of the watershed reflect autochthonous carbon growth.

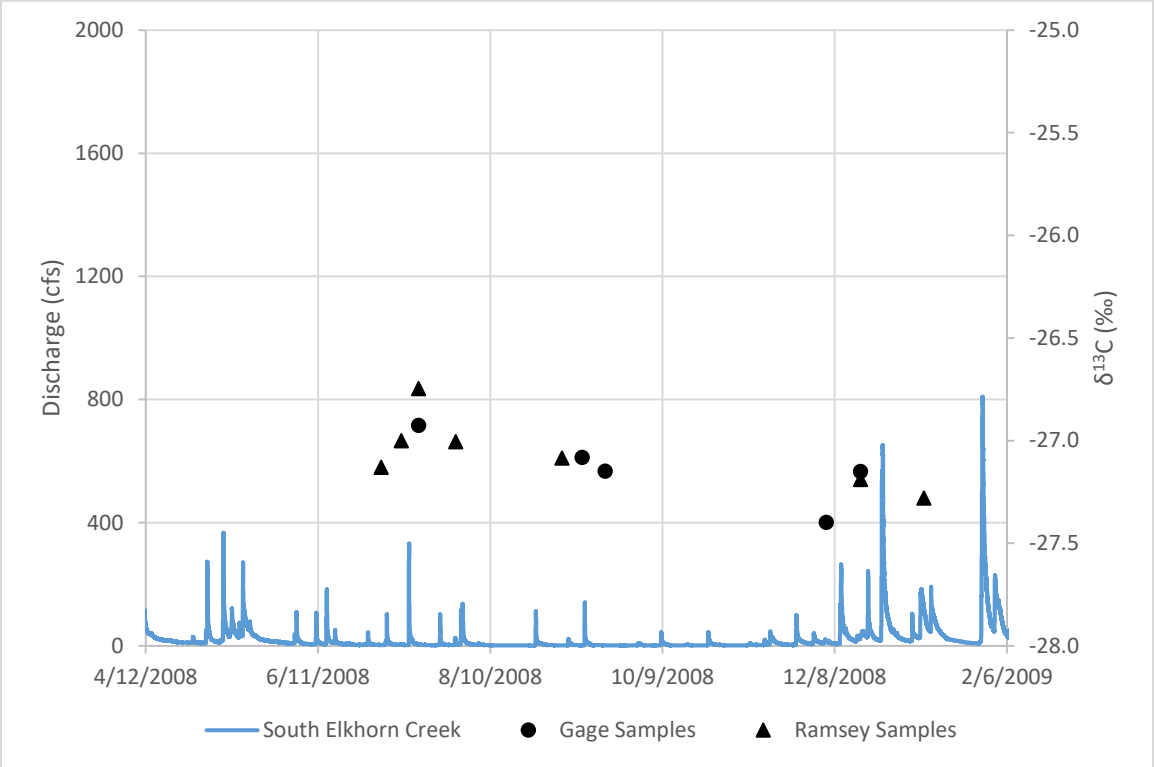


Figure 3-4: Laboratory methods flow chart for batch incubation study

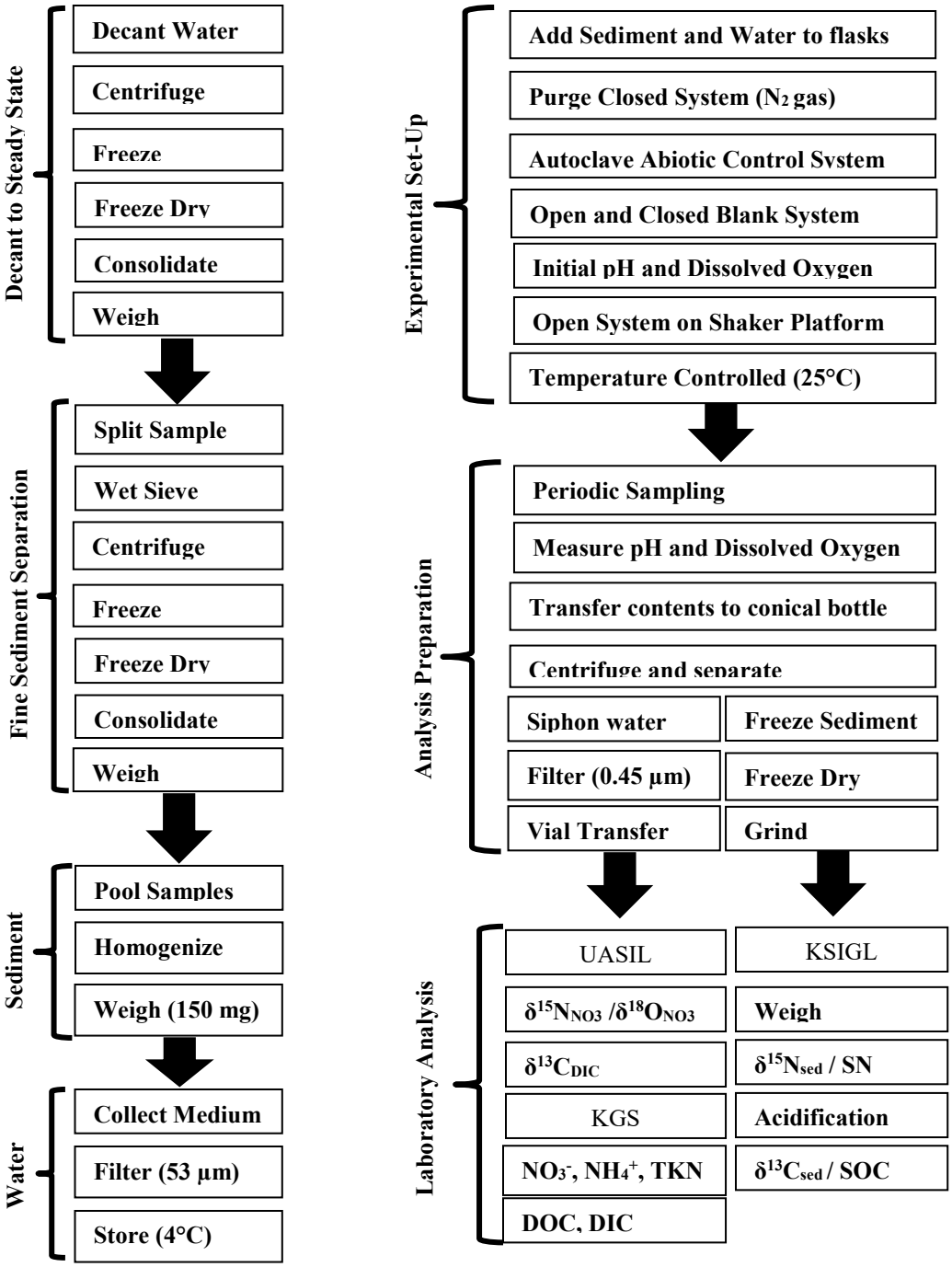


Figure 3-5: Laboratory equipment used in the preparation of fluvial sediment samples

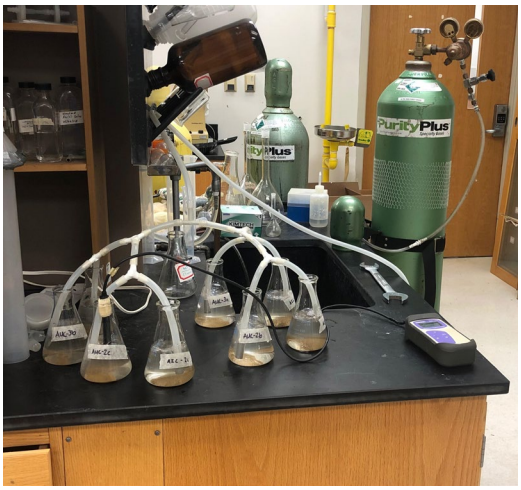
Sorvall® RC-5B Centrifuge

FreeZone® Freeze Dry System

#270 (53-µm) Mesh Sieve

Mortar and pestle

Figure 3-6: Laboratory equipment used in the incubation experiments



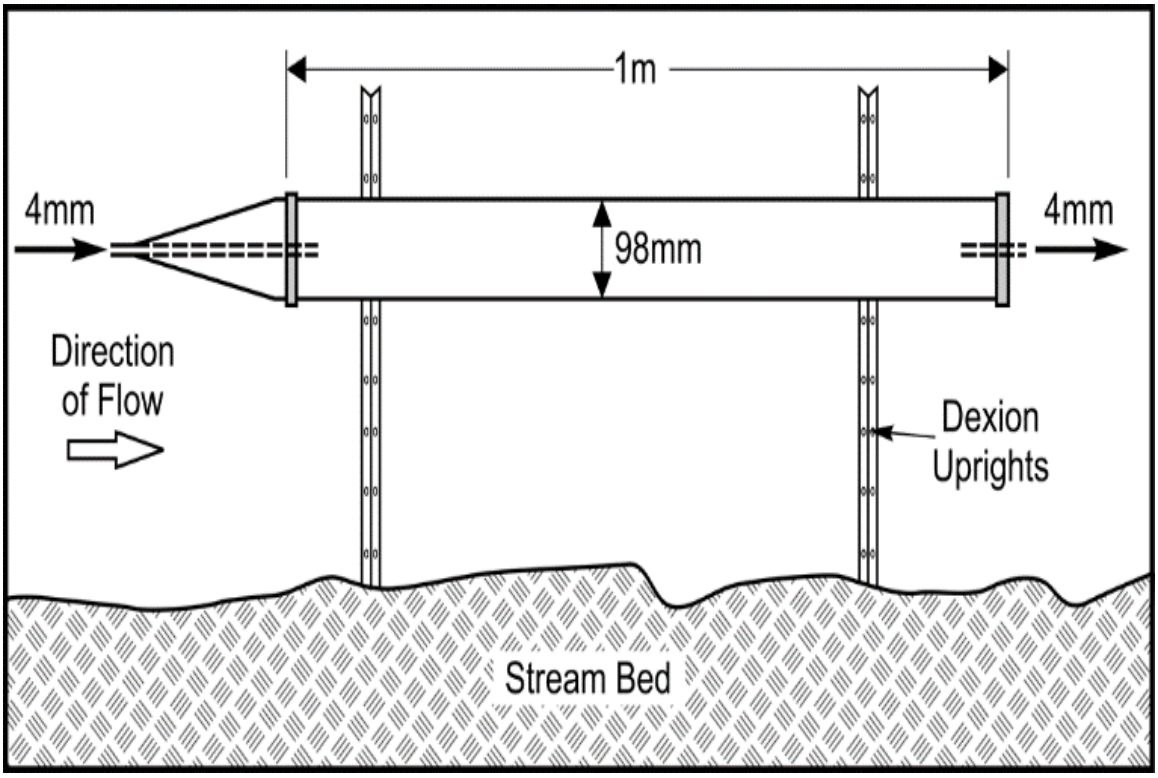
Flasks purged with weak N_2 gas

Erlenmeyer flasks set on orbital shaker platform

HANNA Instruments edge® Dedicated DO Meter

Accumet AB15 pH Meter

Figure 3-7: In-situ sediment trap (Phillips et al., 2000)



Chapter 4 - Data Results from the Laboratory Incubation Study

4.1 Total Nitrogen

Total Nitrogen (TN) was calculated by taking the sum of all measured N compounds (NO_3, NH_3, DON, and SN) in each flask. The mean TN is higher in experimental systems incubated with instream sediment as compared to upland sediment. The open systems had a lower mean TN with upland sediment (0.84 ± 0.04 mg N) than instream sediment (1.02 ± 0.03 mg N). Closed systems had a TN of (0.91 ± 0.09 mg N) and (1.20 ± 0.14 mg N) for upland and instream sediment, respectively (see Figures 4-1 and 4-2). Dissolved matter concentrations determined in the open systems were corrected for H_2O loss caused by evaporation.

Observed differences in TN between systems incubated with upland versus instream sediment is largely attributed to the initial nitrogen pool within the sediments (see Table 4-1). Sediment categorized as originating from uplands is measured to have a smaller nitrogen component (SN = 0.19%) as compared to the identified instream sediment (SN = 0.35%) (see Table 4-1). Results suggest the amount of potentially bioavailable nitrogen within instream sediment leads to an increased production of inorganic nitrogen, in the form of ammonia, as compared to upland sediment (see Table 4-2). Flasks incubated with instream sediment were observed to have an increased concentration of ammonia (NH_3-N = 0.246 – 0.310 mg N l^{-1}) as compared to flasks with upland sediment (NH_3-N = 0.128 – 0.170 mg N l^{-1}) after 7 days (see Table 4-2, Figures 4-1 and 4-2). The observed amount of total nitrogen measured after 70 days of incubation also supports that the initial bioavailability of sediment is important for the production of inorganic nitrogen, and therefore total nitrogen (see Table 4-4). Flasks with upland

sediment has less than one mg of N (OU = 0.805 ± 0.017, AU = 0.829 ± 0.028), and flasks with instream sediment (OI = 0.999 ± 0.009, AI = 1.137 ± 0.024) has about one mg of N through 70 days of incubation. The observed difference in TN for open versus closed systems is largely attributed to water loss that occurs in flasks open to the environment. Measured concentrations were adjusted to account for water loss in the open systems.

Results of TN show that in general nitrogen was balanced in the flasks and the incubation study can be treated as a closed system for nitrogen. There is some variability of TN, especially for the closed flasks, and likely this variability is arriving from the TKN measurements that were used to estimate dissolved organic nitrogen. Nevertheless, the data results for TN give us confidence in estimating reaction rates for the flasks.

4.2 Total Carbon

Total Carbon (TC) was calculated by taking the sum of all measured C compounds (DIC, DOC, and SOC) in each flask. Through 70 days of incubation the open systems had a lower mean TC with upland sediment (8.78 ± 0.49 mg C) than instream sediment (12.23 ± 0.20 mg C). Closed systems had a TC of (13.84 ± 0.72 mg C) and (16.87 ± 0.33 mg C) for upland and instream sediment, respectively (see Table 4-4, Figures 4-3 and 4-4). Total C is expected to be less in the open systems as compared to the closed systems due to loss of C via CO_2 evasion. Dissolved organic carbon (DOC) and sediment organic carbon (SOC) are both decreasing throughout the study. Data results suggest that organic carbon may be decomposed (oxidized) and released as dissolved inorganic carbon (DIC) in the form of CO_2.

The experimental results may be treated as a closed system and open system for carbon for the closed and open flasks, respectively. Results of TC show that in general carbon was balanced in the closed flasks and the closed flask incubation study can be treated as a closed system for carbon. There may be some initial CO_2 evasion in the closed flasks and for example the upland closed flasks show this possibility. However, in general, carbon is balanced giving us confidence in estimating reaction rates for the flasks. Results of TC for the open flasks show that TC linearly decreases over time, albeit variability. The reason for this open system is because carbon is lost to the atmosphere due to CO_2 evasion. Therefore, this must be treated as an open system and evasion must be considered in any carbon mass balance modelling. There is variability of TC for all experiments. This likely mostly comes from measurements of DOC, which had high variability in this study.

4.3 Ammonia

Initial ammonia is minimal in the South Elkhorn stream water used during incubations (< 0.05 mg N l^{-1}). Potential reasons are: NH_3 entering streams maybe rapidly removed from the water by biological assimilation, sorption, and nitrification (Birgand et al., 2007), NH_3 concentrations are typically lower than NO_3 concentrations because NO_3 inputs are often higher than NH_3 inputs and more readily sorbs to sediments (Peterson et al. 2001). Further, microbes preferably assimilate NH_3 to NO_3 due to the energetic gain, and NH_3 is rapidly nitrified (Peterson et al., 2001; Ford et al., 2017).

A sharp ammonia concentration increase is shown (see Figure 4-T1) after 3 days of experimental incubation in all biotic systems with sediment. Open and closed systems incubated with upland sediment increase to about 0.1 mg N l^{-1} (0.116 – 0.133 mg N l^{-1}),

while systems with instream (more labile organic matter) sediment increases to about 0.2 mg N l^{-1} (0.222 – 0.23 mg N l^{-1}). The difference in sediment organic matter content and initial ammonia increase suggests a portion of organic nitrogen undergoes enzymatic hydrolysis when sediment is initially introduced to water.

Differences in the initial minimal ammonia concentrations (< 0.005 mg N l^{-1}) and the maximum ammonia concentration after a week of incubation (see Figure 4-T1) may be attributed to the release of ammonia from the hydrolysis of sediment organic nitrogen. Data results provide evidence oxidation of the released ammonia begins after the first week of incubation, and is utilized by ammonia oxidizing microorganisms throughout the study. There is minimal (≤ 0.05 mg N l^{-1}) or a non-detectable amount of ammonia throughout the remaining incubation period. Due to the results that suggest hydrolysis occurs between day zero and day three, we suggest all modelling of reactions with ammonia start on day 3. This was carried forward to our mass balance and reaction modelling.

4.4 Nitrate

Nitrate is increasing throughout studies incubated in the laboratory (see Figure 4-T3). Nitrate increase most of all in studies with sediment, for almost all days for almost all sediment types and flask types (i.e., open, closed). This supports the idea that some sediment nitrogen is being mineralized to NH_4, then nitrified to nitrate. Nitrate increases are greater in flasks with sediment as compared to those with just water. Nitrate also increases in flasks with just water, referred to as blank controls, however this is expected because initial NH_4 will be converted to nitrate via nitrification and DON will be mineralized and nitrified as well.

We seemed to have a problem in the experiment with the abiotic controls and we believe these controls were not truly abiotic. Sterilized control systems have a mass of nitrate less or equivalent to the full study, generally supporting that some amount of nitrate is being generated via biotic production, i.e., nitrification. Increasing nitrate in the sterilized studies is questionable, as theoretically all N transforming bacteria should be inactive, and therefore nitrate concentrations are expected to remain stable or to decrease via abiotic N removal such as sorption to sediments. Therefore, we believe that we incorrectly sterilized the samples, and the samples were not fully sterilized; thus some biotic processes did exist in the flasks. This could have been due to contamination of the flasks, biota/microbes introduced by the sediment, or incomplete sterilization.

4.5 Dissolved Inorganic Carbon and pH

DIC occurs as CO_3^{2-}, HCO_3^-, H_2CO_3, and dissolved CO_2, collectively forming the carbonate system (Jensen et al., 2018). Data results suggest the main factor affecting DIC concentrations in the incubation study is whether the flasks are open to the atmosphere or closed with a rubber stopper. Data resulting from the open system incubations suggest that evasion, or outgassing of CO_2, is an important factor controlling observed DIC. A decrease in DIC (Figure 4-5) and a decrease in total carbon (Figure 4-3) are observed in the open incubations. $\delta^{13}C_{DIC}$ in these systems are rapidly enriched from an initial value of -11‰ to -4‰ after 14 days of incubation (see Table 4-2, Figure 4-9). After the initial outgassing period, the isotopic signature is slightly enriched to about -2‰ after 70 days of incubation. Data results suggest as CO_2 is outgassed the $\delta^{13}C_{DIC}$ moves towards equilibrium with the atmosphere.

DIC concentrations are relatively stable in closed flasks, and $\delta^{13}C_{DIC}$ is becoming more negative throughout the incubation (see Table 4-2). The $\delta^{13}C_{DIC}$ signature may be decreasing because a source of DIC comes from the decomposition of dissolved organic carbon (DOC) and sediment organic carbon (SOC). CO_2 is respired by microbial organisms that use organic matter as an energy source (Allan, 2007). The $\delta^{13}C$ signature of the sediment is about -27‰, therefore it is a possibility the ^{13}C atoms being decomposed are contributing to the DIC pool and depleting its isotopic value.

The initial pH of the incubated flasks were relatively consistent throughout all systems (pH = 7.59 – 7.72). There is a differing trend in the pH observed in the open and closed systems. The pH increases to about 8.2 in the open flasks, and decreases to about 7.3 in the closed flasks (see Figure 4-T16). It is known that both biological and physical processes can affect stream chemistry, and therefore pH. If water contains elevated concentrations of carbon dioxide (CO_2) the degassing process will affect water chemistry (Choi et al., 1998). A potential explanation for the differing pH in the two types of flask is attributing the evasion process to be a substantial mechanism impacting the water's alkalinity.

4.6 Sediment Organic Matter- SOC and SN

The oxidation of sediment organic carbon (SOC) and subsequent mineralization of nitrogen (SN) was suggested to occur based on observations in all biotic incubated systems with sediment. The two main drivers of sediment organic matter being released as an inorganic form is the availability of labile organic matter and its oxygen demand. Open flasks were measured to have a moderate dissolved oxygen concentration (DO-O= 6.53 ± 1.03 mg l^{-1}) and closed flasks with a low dissolved oxygen concentration (DO-O=

2.61 ± 1.65 mg l^{-1}). Flasks with the more labile instream sediment incubated in an open environment had the greatest reduction (24.76%) of SN from the initial value through 70 days (see Figure 4-T6). The open instream sediment corresponded with a SOC degradation of 8.78% from its initial value. The closed system with the more recalcitrant upland sediment had the lowest reduction (7.02%) from initial SN, and was observed to lose SOC by 5.43%. The SOC reduction was smallest in the closed flask with instream sediment (3.58%), and largest in the open flask with upland sediment (12.73%). SN reduction from its initial value in the open system with upland sediment (17.54%) and the closed system with instream sediment (15.24%) were relatively similar in terms of rate of degradation. In general sediment organic carbon degradation was greater in the open flasks as compared to the closed flasks.

The amount of SOC loss is greater than the loss of SN, although the percent loss of SN is greater than that observed for loss of SOC. For example, SN is observed to reduce by 24.76% in the open system with instream sediment while SOC is only reduced by 8.76% through 70 days. This reduction results from SN losing 0.13 mg through 70 days (0.525 mg to 0.395 mg), while SOC loses 0.49 mg (5.58 mg to 5.09 mg). Although the percent reduction is about three times greater for sediment nitrogen, the observed degradation of SOC is more than three times greater in terms of mass. The different loss rates for SOC and SN within the instream sediment may be attributed to multiple pools of organic matter within the substrate. An algal component of the instream sediment is thought to be a labile, more easily decomposed portion of the fluvial sediment as compared to any deposited terrestrial soil. It is thought the chemical composition and respective contributions of these pools within the sediment is responsible for

stoichiometric differences in the oxidation of organic carbon and mineralization of nitrogen.

4.7 Dissolved Organic Matter – DON and DOC

Dissolved organic matter is decreasing throughout the initial 70-day incubation period (see Figures 4-T2 and 4-T8). The oxidation of the dissolved organic carbon (DOC) pool occurs in all experimental systems, with the amount loss greater in open flasks as compared to closed. It was observed over the 70-day incubation that DOC content is lost at 51-60% of its initial concentration in the open systems, while only 25-34% is lost in closed systems. These results indicate the amount of DOC oxidized during incubation was impacted by the availability of dissolved oxygen, similarly to the oxidation of sediment organic carbon (SOC). Further the oxidation of DOC within experiments supports the two organic matter pools theory as the rate of oxidation is significantly faster during the initial 4 weeks of incubation as compared to the following incubations. It is suspected a reactive pool of the organic matter is susceptible to oxidation and preferentially decomposed. Eventually the reactive material has gone through a stage of decomposition and is then oxidized concurrently with the portion of dissolved organic matter resistant to decomposition.

Results suggest microbes can utilize the dissolved organic nitrogen (DON) portion of the measured Total Kjeldahl Nitrogen (TKN). DON may be ammonified and converted to NH_4 which can then be further oxidized by nitrifiers. The labile portion of the DON maybe rapidly ammonified within the first couple weeks of incubation, corresponding to the decreasing C/N ratio (DOC/DON) observed within the incubation experiments (see Figure 4-T13). It is observed the DON increases near the end of the

experiment from day 70 to 140 of the incubations. A potential reason is due to microbial biomass accumulating in the flasks over the incubation period. TKN measurements may be underestimated because of analysis preparation. TKN samples were split using a 0.45 μm filter, therefore particles of organic nitrogen larger than the filter are not accounted for in analysis. The high variability of measured TKN, and therefore calculated DON, requires careful consideration when interpreting the C/N ratios (see Table 4-1, Figure 4-T2).

4.8 Stable Nitrogen Isotopes - $\delta^{15}N_{NO3}$ and $\delta^{15}N_{sed}$

The initial $\delta^{15}N_{NO3}$ incubated within the open and closed systems were 7.51 ± 0.02‰ (n=3) and 7.45 ± 0.08‰ (n=3) respectively (see Table 4-1). The initial $\delta^{15}N$ values of total nitrogen in the upland (5.94‰) and instream (6.27‰) sediment fall within the typical range for soil organic nitrogen (0 - +7‰) reported by Kendall et al., 2007. Within the following 70 days of the open incubations, the $\delta^{15}N_{NO3}$ value decreased to 6.24‰ and 6.49‰, merging towards the reported $\delta^{15}N$ of sediment (5.98‰ and 6.10‰) after ten weeks (see Figure 4-7).

The difference in $\delta^{15}N$ of sediment is small over the 70-day incubation period, supporting the idea that isotope fractionation occurs during the nitrification step, as opposed to ammonification. The observed data may suggest the fractionation occurring during sediment ammonification is within range of literature reported enrichment values (Prokopenko et al., 2006; Mobius, 2013).

The $\delta^{15}N_{NO3}$ after 10 weeks in the closed incubation systems were decreased to an isotopic signature below the corresponding $\delta^{15}N$ of sediment measured (see Figure 4-8). The closed systems $\delta^{15}N_{NO3}$ were (5.86‰ and 4.36‰) for upland and instream sediment

incubations. Data results indicate the source of ^{15}N for nitrate production comes from an isotopically depleted pool compared to the sediment nitrogen. Literature values suggest $\delta^{15}N_{NH4}$ and $\delta^{15}N_{DON}$ can be isotopically lighter ($\delta^{15}N_{NH4}$ = -3 ± 7‰), ($\delta^{15}N_{DON}$ = +5 ± 3‰) as compared to the measured values of $\delta^{15}N_{NO3}$ and $\delta^{15}N_{Sed}$ (Kendall et al., 2007; Husic et al., 2020). The shift towards lighter $\delta^{15}N_{NO3}$ indicates that DON, SN, and NH_3 with isotopically lighter $\delta^{15}N$ are oxidized to NO_3^-. Further, previous modeling results indicate that the nitrification of isotopically lighter ammonium ($\delta^{15}N_{NH4}$) acts as a mechanism for an increase in NO_3^- that coincides with a decrease in $\delta^{15}N_{NO3}$ (Husic et al., 2020). Data results are in agreement with previous systems where limited ammonia availability governs the mineralization process in which the isotopic signature of nitrate is similar to the $\delta^{15}N$ values of its original organic matter source.

4.9 Stable Carbon Isotopes - $\delta^{13}C_{DIC}$ and $\delta^{13}C_{sed}$

The initial $\delta^{13}C_{DIC}$ incubated within the open and closed systems were -11.72 ± 0.07‰ (n=3) and -9.73 ± 0.20‰ (n=3) respectively (see Table 4-1). The initial $\delta^{13}C$ values of organic carbon in the upland (-26.46‰) and instream (-27.02‰) sediment reflects its chemical composition as the upland sediment is considered to have more humified matter (SOC = 1.78%) and the instream sediment is thought to have accumulated labile algal material (SOC = 3.72%). The $\delta^{13}C_{DIC}$ signature within closed flasks becomes more negative and trends toward the $\delta^{13}C$ values of the incubated organic matter. It is thought the ^{13}C atoms decomposed from the sources of organic matter are contributing to the $\delta^{13}C$ signature observed in the dissolved inorganic carbon phase. The $\delta^{13}C_{DIC}$ within open flasks deviates from the trend observed in the closed flasks. $\delta^{13}C_{DIC}$ in these systems are rapidly enriched from an initial value of -11‰ to -4‰ after 14 days

of incubation (see Table 4-2, Figure 4-9). After the initial outgassing period, the isotopic signature is slightly enriched to about -2‰ after 70 days of incubation. The data results of DIC and $\delta^{13}C_{DIC}$ in the open flasks suggest as CO_2 is outgassed the $\delta^{13}C_{DIC}$ moves towards a more positive delta value reflecting an isotope signature of a system in equilibrium with the atmosphere.

Table 4-1: Initial Laboratory Incubation data results

OB represents the blank control flasks open to the atmosphere. AB is the blank control flasks closed with a rubber stopper. U-0 is the representative upland sediment pool. I-0 is the instream sediment pool.

Sample Lab ID	Ammonia NH_3-N mg N/L	Kjeldahl Nitrogen TKN-N mg N/L	DON-N[a] mg N/L	Nitrate NO_3^-N mg N/L	$\delta^{15}N_{NO3}$ ‰	$\delta^{18}O_{NO3}$ ‰
OB-0 (n=3)	0.005 ± 0.004	0.323 ± 0.230	0.318 ± 0.234	2.230 ± 0.008	7.514 ± 0.022	4.250 ± 0.384
AB-0 (n=3)	0.033 ± 0.005	0.647 ± 0.038	0.613 ± 0.033	2.279 ± 0.035	7.452 ± 0.076	5.654 ± 0.523

Initial dissolved nitrogen elemental and isotopic values measured in the laboratory.

Sample Lab ID	DOC mg C/L	DIC mg C/L	$\delta^{13}C_{DIC}$ ‰
OB-0 (n=3)	16.4 ± 4.576	44 ± 0.816	-11.716 ± 0.069
AB-0 (n=3)	17.767 ± 1.799	40.667 ± 0.471	-9.733 ± 0.196

Sample Lab ID	$\delta^{15}N_{Sed}$ ‰	SN %	$\delta^{13}C_{Sed}$ ‰	SOC %
U-0	5.94	0.19	-26.46	1.78
I-0	6.27	0.35	-27.02	3.72

Initial dissolved carbon and sediment elemental and isotopic values measured in the laboratory.

Flask Label	DO mg/L	pH	Temp °C
Open	6.53 ± 1.03	7.70 ± 1.38	25.1 ± 0.3
Closed	2.61 ± 1.65	7.40 ± 1.35	25.1 ± 0.3

Experimental data results including dissolved oxygen, pH, and temperature.

Table 4-2: Laboratory Incubation water and sediment data results

Dissolved nitrogen elemental and isotopic values measured in the laboratory.

Sample Lab ID	Time	Ammonia NH_3-N	Kjeldahl Nitrogen TKN-N	DON-N[a]	Nitrate NO_3^--N	$\delta^{15}N_{NO3}$	$\delta^{18}O_{NO3}$
	days	mg N/L	mg N/L	mg N/L	mg N/L	‰	‰
OU-1*	3	0.116	0.613	0.497	2.150	7.550	3.662
OU-2	7	0.128	0.630	0.502	1.870	6.995	2.924
OU-3	14	0.019	0.737	0.718	1.987	7.085	1.223
OU-4*	21	0.007	0.318	0.311	2.410	7.430	3.027
OU-5	28	0.019	< MDL	0[b]	2.698	7.353	2.759
OU-6	42	0.018	0.400	0.382	2.674	7.500	2.471
OU-7	56	< MDL	0.378	0.378	2.665	6.839	2.194
OU-8*	70	< MDL	< MDL	0.000	2.849	6.241	2.559
OU-9	140	0.005	0.357	0.352	2.706	5.967	1.671
OI-1*	3	0.222	0.732	0.510	2.175	7.543	4.218
OI-2	7	0.246	0.690	0.443	1.966	7.169	2.727
OI-3	14	0.019	0.592	0.572	2.067	7.297	-0.786
OI-4*	21	0.013	0.296	0.283	2.612	7.707	2.502
OI-5	28	0.008	< MDL	0[b]	2.826	7.427	1.736
OI-6	42	0.009	< MDL	0[b]	2.961	7.205	1.467
OI-7	56	< MDL	< MDL	0.000	2.625	6.672	0.391
OI-8*	70	< MDL	< MDL	0.000	3.022	6.487	1.575
OI-9	140	0.003	0.322	0.319	2.706	6.369	0.526
AU-1*	3	0.133	0.603	0.470	3.322	7.422	5.459
AU-2	7	0.170	0.620	0.450	1.968	7.503	3.967
AU-3	14	0.030	0.680	0.650	2.131	6.345	0.988
AU-4*	21	0.013	0.637	0.623	2.494	6.926	2.053
AU-5	28	0.020	0.520	0.500	2.531	6.722	2.782
AU-6	42	0.010	< MDL	0[b]	2.848	6.412	2.561
AU-7	56	< MDL	< MDL	0.000	3.322	5.370	0.641
AU-8*	70	< MDL	< MDL	0.000	2.818	5.856	2.652
AU-9	140	< MDL	0.530	0.530	3.345	3.246	0.257
AI-1*	3	0.230	1.183	0.953	3.277	7.918	4.815
AI-2	7	0.310	0.550	0.240	2.034	7.349	3.341
AI-3	14	0.020	0.750	0.730	2.034	6.880	-1.091
AI-4*	21	0.020	0.493	0.473	2.840	6.954	2.412
AI-5	28	0.050	0.840	0.790	3.955	5.209	1.293
AI-6	42	0.006	0.490	0.484	3.051	5.401	0.146
AI-7	56	< MDL	0.440	0.440	3.254	5.613	1.037
AI-8*	70	< MDL	< MDL	0.000	3.458	4.362	0.569
AI-9	140	0.050	0.980	0.930	3.322	5.260	1.152

[a] DON-N calculated using the following equation: DON = TKN - NH_3. If both TKN and NH_3 are < MDL, DON = 0.

[b] Calculated DON-N values < 0 are reported as 0.

< MDL: Values measures below the minimum detection limit are reported as 0.

* Samples are triplicated and measured separately. Mean values of the triplicate is reported.

Dissolved C elemental and isotopic values measured in the lab. Sediment elemental and isotopic values measured.

Sample	Time	DOC	DIC	$\delta^{13}C_{DIC}$	$\delta^{15}N_{Sed}$	SN	$\delta^{13}C_{Sed}$	SOC
	days	mg C/L	mg C/L	‰	‰	%	‰	%
OU-1*	3	17.553	39.743	-9.296	5.83	0.19	-26.47	2.02
OU-2	7	14.874	28.565	-5.916	5.80	0.19	-26.61	1.73
OU-3	14	11.931	32.010	-4.146	5.79	0.19	-26.48	1.89
OU-4*	21	3.120	37.372	-2.822	5.65	0.18	-26.40	1.90
OU-5	28	3.478	31.960	-2.694	6.06	0.18	-26.16	1.72
OU-6	42	10.101	28.210	-2.319	6.24	0.19	-26.32	1.73
OU-7	56	7.244	26.400	-2.612	6.24	0.21	-26.28	1.62
OU-8*	70	6.458	25.783	-2.422	5.98	0.16	-26.43	1.55
OU-9	140	5.600	21.700	-1.743	5.47	0.17	-26.50	1.73
OI-1*	3	18.878	42.392	-9.532	6.27	0.33	-27.13	3.91
OI-2	7	16.056	35.460	-5.506	5.86	0.32	-27.15	3.61
OI-3	14	12.125	33.950	-4.010	5.91	0.32	-27.02	3.72
OI-4*	21	3.629	42.529	-3.277	6.02	0.31	-26.84	4.11
OI-5	28	9.400	42.300	-3.100	6.02	0.31	-26.82	3.61
OI-6	42	7.371	30.940	-1.970	6.23	0.30	-27.01	3.51
OI-7	56	9.310	39.600	-2.607	6.12	0.30	-27.01	3.49
OI-8*	70	7.935	27.767	-2.240	6.10	0.26	-27.10	3.39
OI-9	140	1.610	23.800	-1.852	5.63	0.27	-26.96	3.31
AU-1*	3	21.633	42.000	-10.694	5.67	0.19	-26.53	2.07
AU-2	7	15.300	39.000	-10.843	5.48	0.20	-26.58	1.94
AU-3	14	13.600	36.000	-11.580	5.35	0.19	-26.61	2.06
AU-4*	21	4.467	44.400	-11.267	5.92	0.18	-26.15	1.46
AU-5	28	10.500	44.000	-10.869	5.83	0.20	-26.40	1.73
AU-6	42	8.900	44.000	-11.705	5.99	0.19	-26.52	1.91
AU-7	56	13.740	35.000	-12.238	5.91	0.18	-26.45	1.75
AU-8*	70	13.223	43.333	-12.897	5.98	0.18	-26.64	1.68
AU-9	140	8.300	44.000	-11.741	5.48	0.17	-26.52	1.89
AI-1*	3	20.367	42.333	-10.498	6.14	0.32	-27.10	3.58
AI-2	7	16.100	43.000	-11.330	5.77	0.33	-27.15	3.76
AI-3	14	9.000	29.000	-11.340	5.97	0.32	-27.05	3.69
AI-4*	21	4.400	44.400	-11.392	5.78	0.31	-26.75	3.79
AI-5	28	5.200	57.000	-11.557	5.93	0.33	-26.96	3.74
AI-6	42	8.900	45.000	-12.075	6.10	0.31	-27.02	3.51
AI-7	56	14.970	44.000	-11.161	5.97	0.30	-27.10	3.57
AI-8*	70	11.790	45.667	-12.443	6.03	0.30	-27.01	3.59
AI-9	140	10.400	46.000	-14.894	5.49	0.31	-27.24	3.75

* Samples are triplicated and measured separately. Mean values of the triplicate is reported.

Table 4-3: Laboratory Incubation control water and sediment data results

Samples with a B are blank creek water flasks. Samples with a C are sterilized flasks to inhibit microbial activity.

Dissolved nitrogen elemental and isotopic values measured in the laboratory.

Sample Lab ID	**Time** days	**Ammonia NH_3-N** mg N/L	**Kjeldahl Nitrogen TKN-N** mg N/L	**DON-N[a]** mg N/L	**Nitrate NO_3^--N** mg N/L	**$\delta^{15}N_{NO3}$** ‰	**$\delta^{18}O_{NO3}$** ‰
OB-0*	0	0.005	0.323	0.318	2.230	7.514	4.250
OB-1	14	0.019	< MDL	0[b]	1.995	7.682	3.057
OB-2	28	< MDL	< MDL	0.000	2.315	7.060	3.009
OB-3	56	< MDL	0.510	0.510	2.645	6.599	2.039
AB-0*	0	0.033	0.647	0.613	2.279	7.452	5.654
AB-1	14	0.010	0.500	0.490	2.167	6.680	2.526
AB-2	28	0.010	< MDL	0[b]	2.599	6.143	3.583
AB-3	56	< MDL	0.430	0.430	2.644	5.281	0.755
OUC-1	14	0.097	0.427	0.330	2.025	7.442	3.361
OUC-2	28	0.122	0.442	0.320	2.485	8.643	4.089
OUC-3*	56	0.066	0.434	0.368	2.645	7.477	3.610
OIC-1	14	0.233	0.640	0.407	1.966	7.624	3.018
OIC-2	28	0.235	0.461	0.226	2.443	8.596	3.744
OIC-3*	56	0.202	0.695	0.493	2.619	7.608	3.762
AUC-1	14	0.160	0.630	0.470	2.011	7.709	4.425
AUC-2	28	0.110	0.610	0.500	2.373	8.451	2.946
AUC-3*	56	0.390	1.117	0.727	2.561	7.263	3.307
AIC-1	14	0.320	< MDL	0[b]	1.993	7.669	4.152
AIC-2	28	0.160	0.730	0.570	2.373	7.617	1.634
AIC-3*	56	0.440	1.063	0.623	2.833	6.836	2.823

[a] DON-N calculated using the following equation: DON = TKN - NH_3. If both TKN and NH_3 are < MDL, DON = 0.

[b] Calculated DON-N values < 0 are reported as 0.

< MDL: Values measures below the minimum detection limit are reported as 0.

* Samples are triplicated and measured separately. Mean values of the triplicate is reported.

Dissolved C elemental and isotopic values measured in the lab. Sediment elemental and isotopic values measured.

Sample	Time	DOC	DIC	$\delta^{13}C_{DIC}$
	days	mg C/L	mg C/L	‰
OB-0*	0	16.400	44.000	-11.716
OB-1	14	13.386	12.610	-1.905
OB-2	28	2.632	15.040	-2.922
OB-3	56	8.437	23.760	-2.648
AB-0*	0	17.767	40.667	-9.733
AB-1	14	9.700	37.000	-10.079
AB-2	28	9.600	40.000	-11.137
AB-3	56	13.080	45.000	-11.484
OUC-1	14	12.222	5.820	-3.697
OUC-2	28	3.854	23.500	-3.560
OUC-3*	56	6.933	24.933	-3.137
OIC-1	14	14.259	24.250	-3.648
OIC-2	28	4.324	23.500	-3.561
OIC-3*	56	6.944	24.640	-2.536
AUC-1	14	11.000	17.000	-9.500
AUC-2	28	5.800	25.000	-9.719
AUC-3*	56	7.180	25.000	-10.313
AIC-1	14	12.900	19.000	-9.744
AIC-2	28	10.000	24.000	-10.195
AIC-3*	56	6.563	23.667	-9.700

Sample	$\delta^{15}N_{Sed}$	SN	$\delta^{13}C_{Sed}$	SOC
	‰	%	‰	%
OB-0				
OB-1				
OB-2				
OB-3				
AB-0				
AB-1				
AB-2				
AB-3				
OUC-1*	6.08	0.19	-26.50	1.65
OUC-2*	6.00	0.18	-26.51	1.56
OUC-3*	6.11	0.18	-26.31	1.77
OIC-1*	6.07	0.32	-27.11	3.47
OIC-2*	6.14	0.32	-27.07	3.65
OIC-3*	6.31	0.30	-26.99	3.64
AUC-1*	5.63	0.20	-26.57	1.67
AUC-2*	5.50	0.20	-26.58	1.77
AUC-3*	4.79	0.20	-26.73	1.94
AIC-1*	5.87	0.34	-27.08	3.82
AIC-2*	6.06	0.34	-27.22	3.79
AIC-3*	5.67	0.33	-27.03	3.79

* Samples are triplicated and measured separately. Mean values of the triplicate is reported.

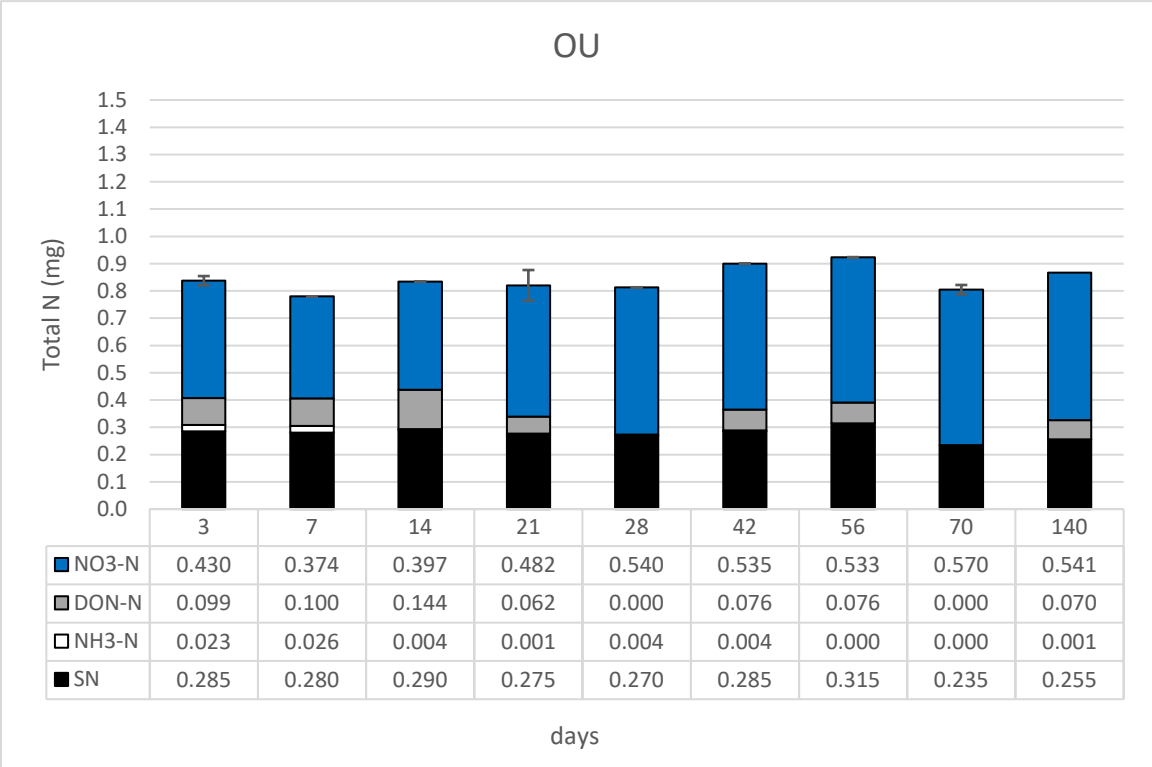
Table 4-4: Total Nitrogen and Carbon in incubation study
Total Nitrogen (N) and Carbon (C) measured at the beginning (day 3) and end (day 70) of the incubation study.

Sample Lab ID	Time	Total Nitrogen	Total Carbon
	days	mg N	mg C
OU-1*	3	0.838 ± 0.017	14.494 ± 0.762
OU-8*	70	0.805 ± 0.017	8.778 ± 0.493
OI-1*	3	1.081 ± 0.042	18.114 ± 0.570
OI-8*	70	0.999 ± 0.009	12.230 ± 0.204
AU-1*	3	1.075 ± 0.187	15.832 ± 0.496
AU-8*	70	0.829 ± 0.028	13.836 ± 0.724
AI-1*	3	1.377 ± 0.077	17.910 ± 0.612
AI-8*	70	1.137 ± 0.024	16.871 ± 0.329

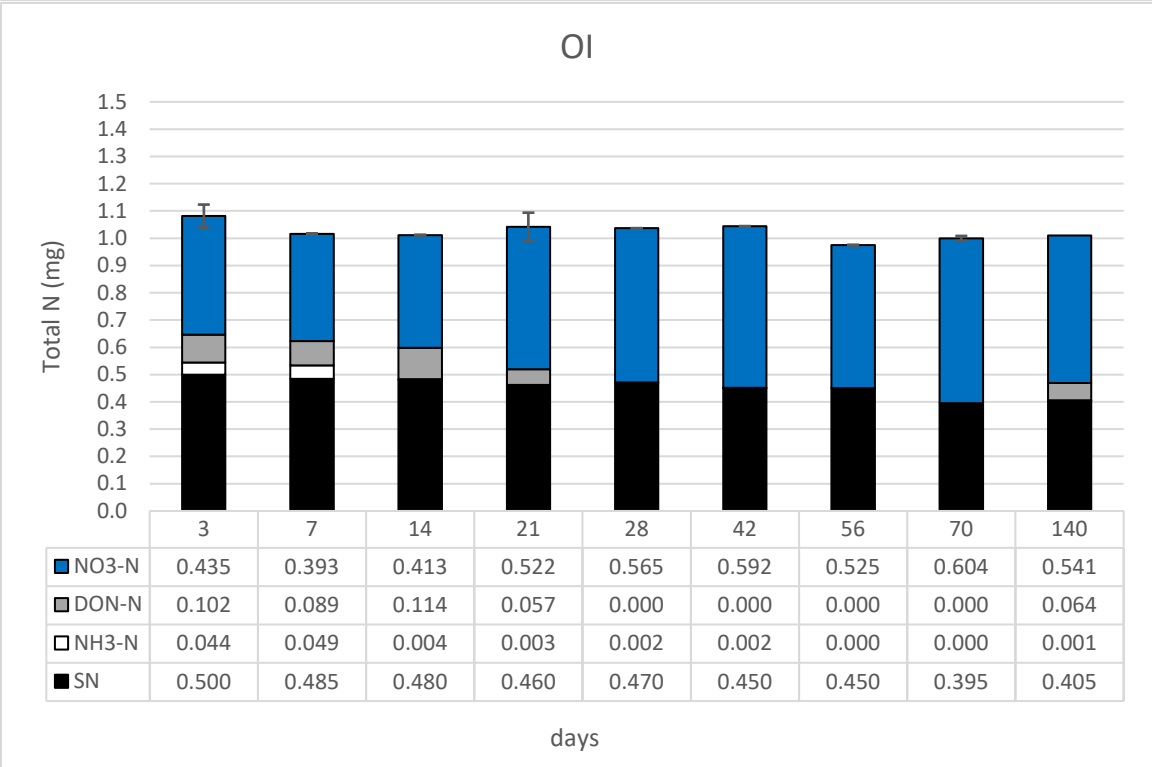
Error is the standard deviation simulated via Monte Carlo analysis (N=1500)

Figure 4-1: Mass of Nitrogen compounds (mg) in each open experimental system

Error bars is the standard deviation of Total N simulated via Monte Carlo analysis. (Only for triplicated analysis)



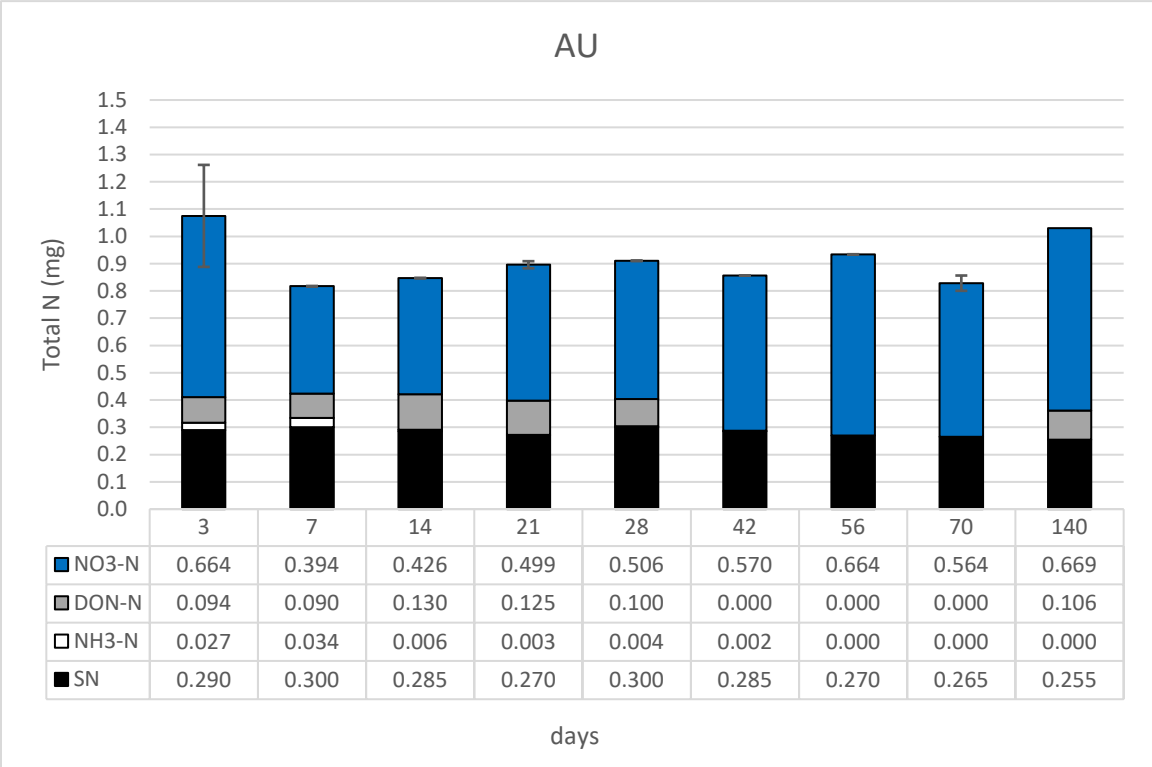
	3	7	14	21	28	42	56	70	140
NO3-N	0.430	0.374	0.397	0.482	0.540	0.535	0.533	0.570	0.541
DON-N	0.099	0.100	0.144	0.062	0.000	0.076	0.076	0.000	0.070
NH3-N	0.023	0.026	0.004	0.001	0.004	0.004	0.000	0.000	0.001
SN	0.285	0.280	0.290	0.275	0.270	0.285	0.315	0.235	0.255



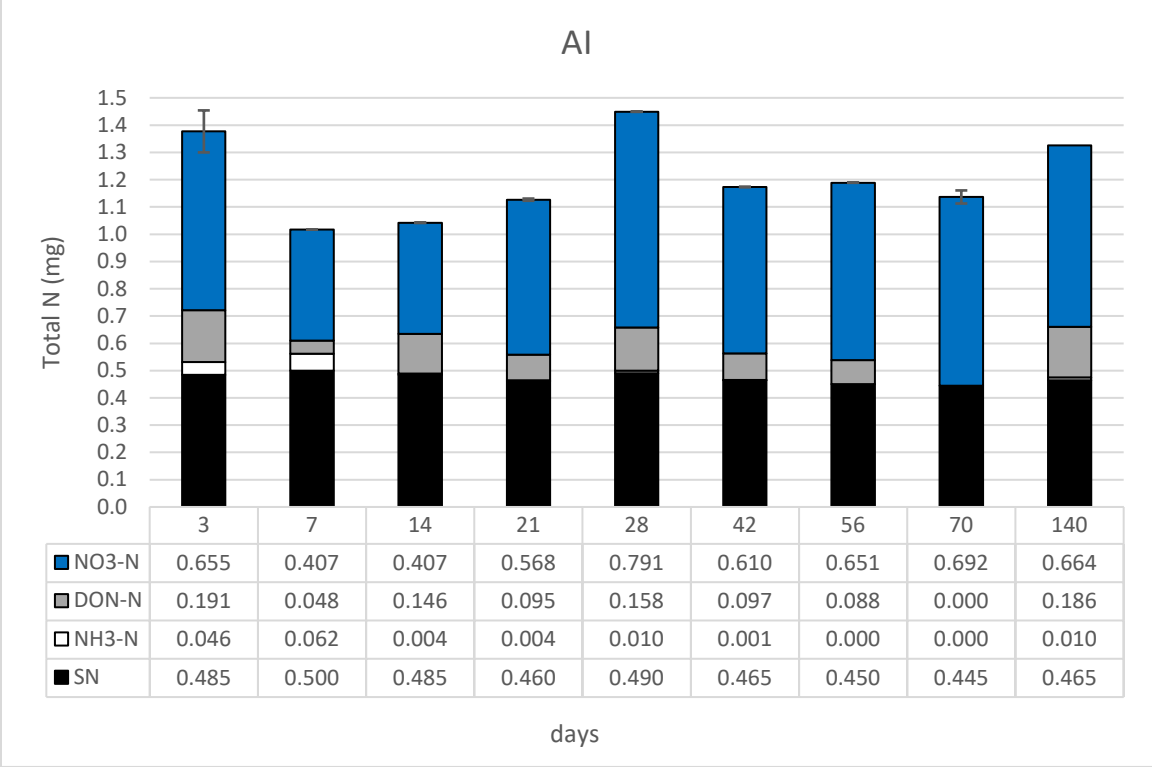
	3	7	14	21	28	42	56	70	140
NO3-N	0.435	0.393	0.413	0.522	0.565	0.592	0.525	0.604	0.541
DON-N	0.102	0.089	0.114	0.057	0.000	0.000	0.000	0.000	0.064
NH3-N	0.044	0.049	0.004	0.003	0.002	0.002	0.000	0.000	0.001
SN	0.500	0.485	0.480	0.460	0.470	0.450	0.450	0.395	0.405

Figure 4-2: Mass of Nitrogen compounds (mg) in each closed experimental system

Error bars is the standard deviation of Total N simulated via Monte Carlo analysis. (Only for triplicated analysis)



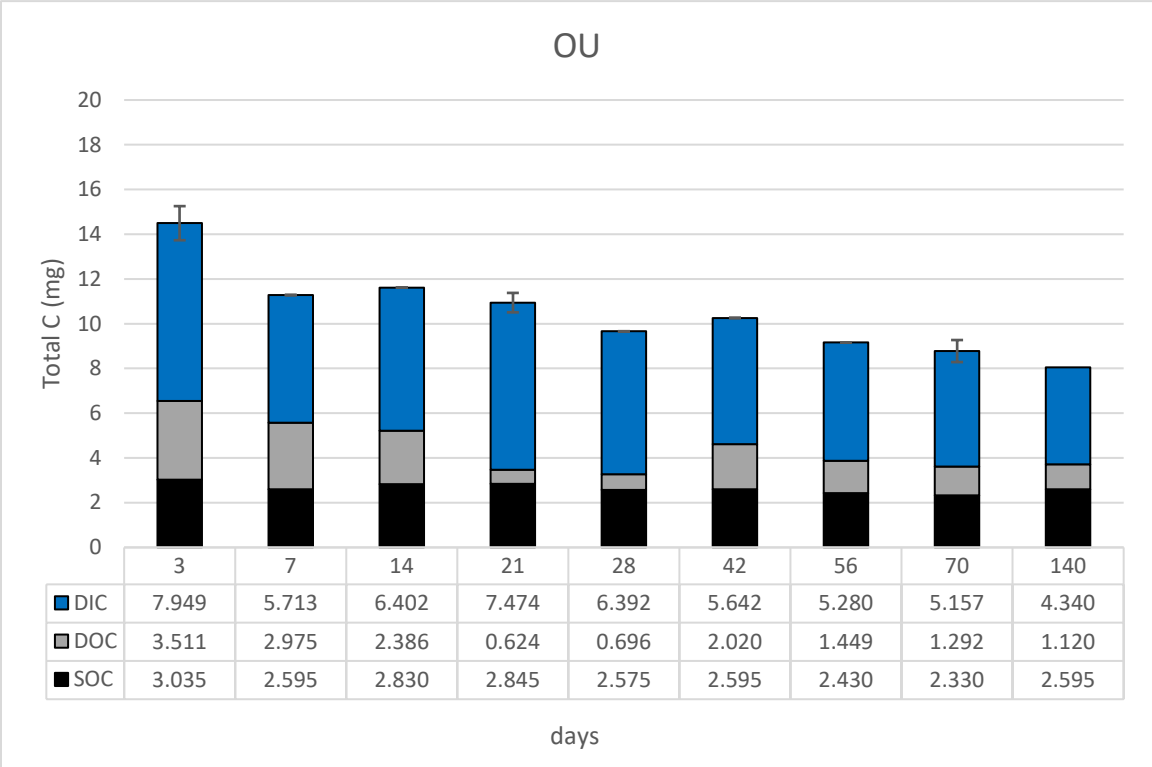
	3	7	14	21	28	42	56	70	140
NO3-N	0.664	0.394	0.426	0.499	0.506	0.570	0.664	0.564	0.669
DON-N	0.094	0.090	0.130	0.125	0.100	0.000	0.000	0.000	0.106
NH3-N	0.027	0.034	0.006	0.003	0.004	0.002	0.000	0.000	0.000
SN	0.290	0.300	0.285	0.270	0.300	0.285	0.270	0.265	0.255



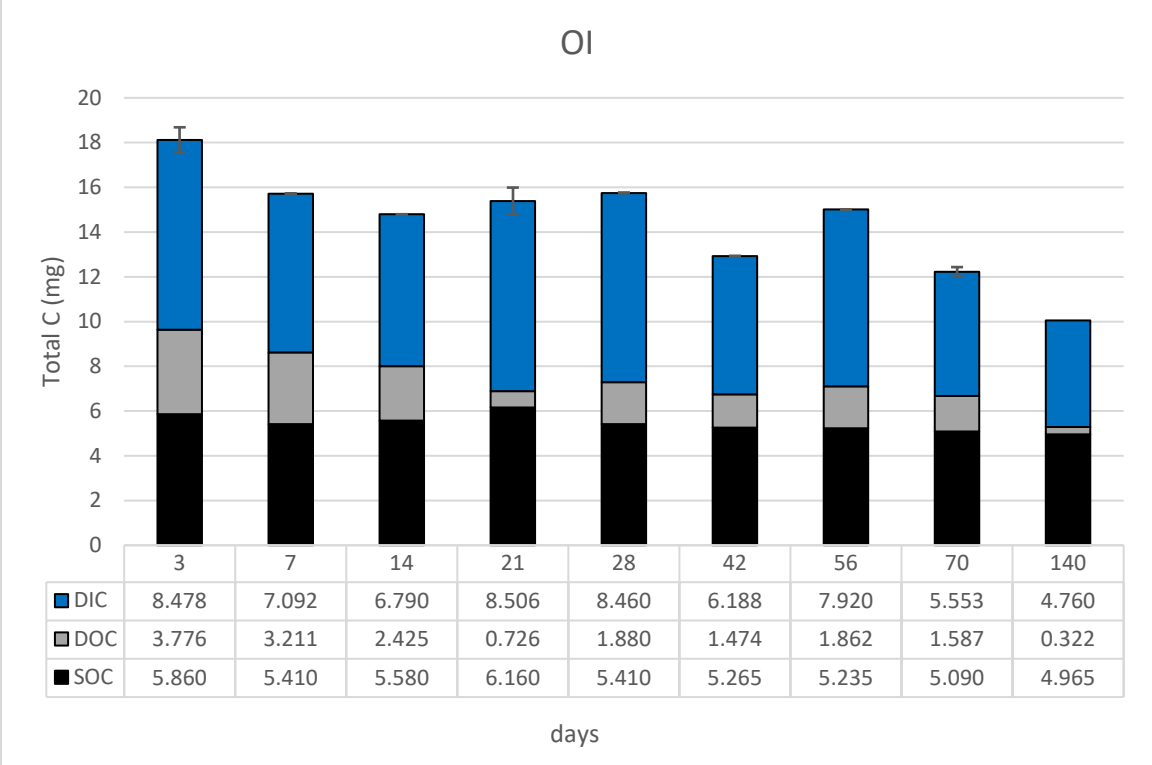
	3	7	14	21	28	42	56	70	140
NO3-N	0.655	0.407	0.407	0.568	0.791	0.610	0.651	0.692	0.664
DON-N	0.191	0.048	0.146	0.095	0.158	0.097	0.088	0.000	0.186
NH3-N	0.046	0.062	0.004	0.004	0.010	0.001	0.000	0.000	0.010
SN	0.485	0.500	0.485	0.460	0.490	0.465	0.450	0.445	0.465

Figure 4-3: Mass of Carbon compounds (mg) in each open experimental system

Error bars is the standard deviation of Total C simulated via Monte Carlo analysis. (Only for triplicated analysis)



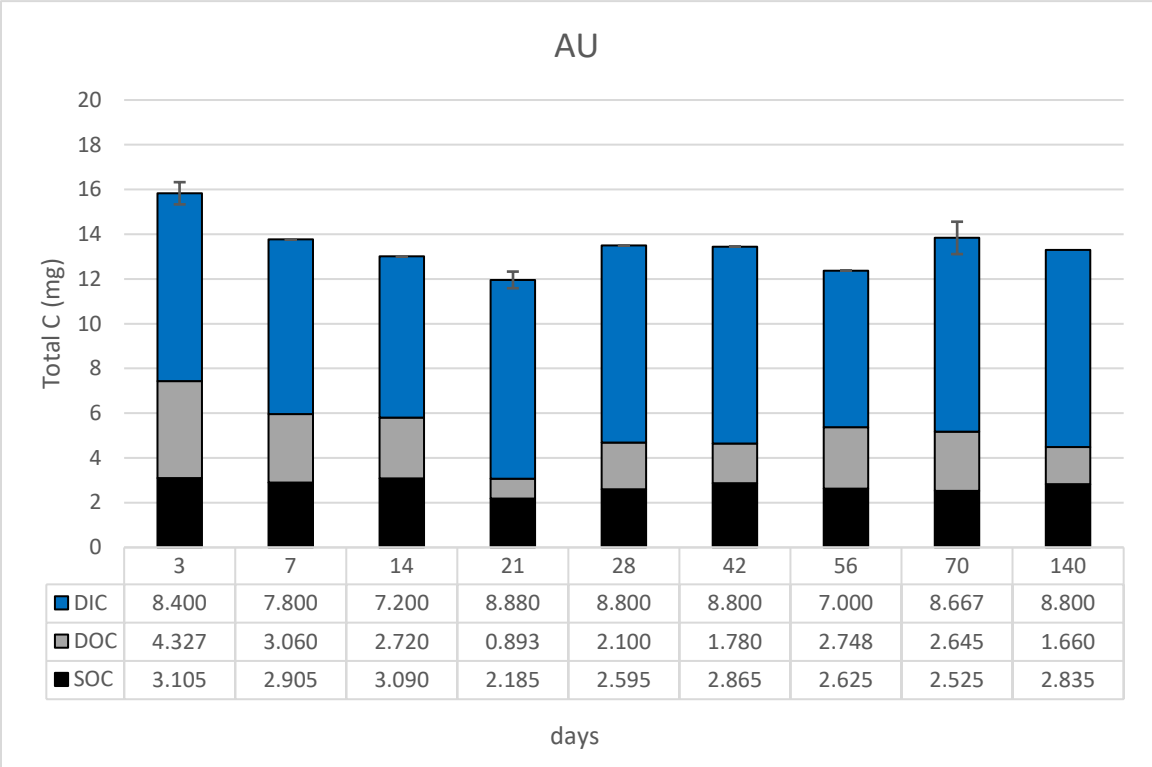
	3	7	14	21	28	42	56	70	140
DIC	7.949	5.713	6.402	7.474	6.392	5.642	5.280	5.157	4.340
DOC	3.511	2.975	2.386	0.624	0.696	2.020	1.449	1.292	1.120
SOC	3.035	2.595	2.830	2.845	2.575	2.595	2.430	2.330	2.595



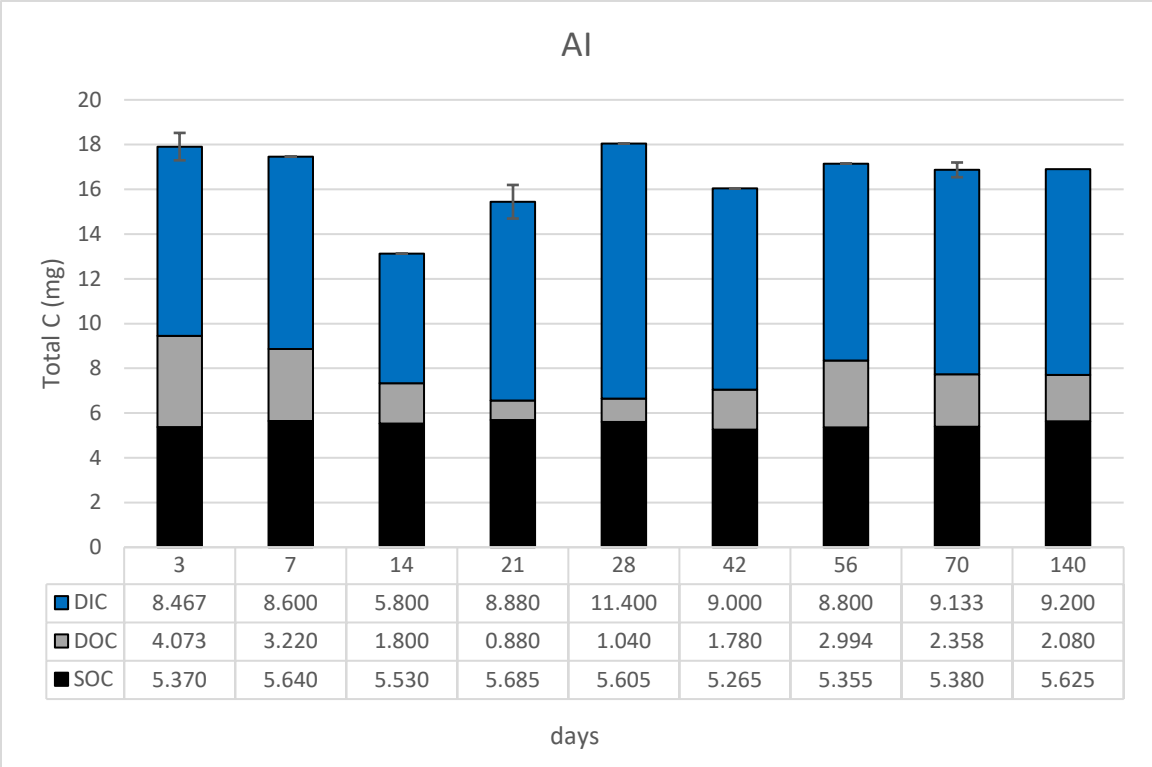
	3	7	14	21	28	42	56	70	140
DIC	8.478	7.092	6.790	8.506	8.460	6.188	7.920	5.553	4.760
DOC	3.776	3.211	2.425	0.726	1.880	1.474	1.862	1.587	0.322
SOC	5.860	5.410	5.580	6.160	5.410	5.265	5.235	5.090	4.965

Figure 4-4: Mass of Carbon compounds (mg) in each closed experimental system

Error bars is the standard deviation of Total C simulated via Monte Carlo analysis. (Only for triplicated analysis)



	3	7	14	21	28	42	56	70	140
DIC	8.400	7.800	7.200	8.880	8.800	8.800	7.000	8.667	8.800
DOC	4.327	3.060	2.720	0.893	2.100	1.780	2.748	2.645	1.660
SOC	3.105	2.905	3.090	2.185	2.595	2.865	2.625	2.525	2.835



	3	7	14	21	28	42	56	70	140
DIC	8.467	8.600	5.800	8.880	11.400	9.000	8.800	9.133	9.200
DOC	4.073	3.220	1.800	0.880	1.040	1.780	2.994	2.358	2.080
SOC	5.370	5.640	5.530	5.685	5.605	5.265	5.355	5.380	5.625

Figure 4-5: NO_3^- and DIC incubated in each open experimental system

Black points denote nitrate (NO_3^-) concentration and grey points denote dissolved inorganic carbon (DIC).Triangles are control systems.

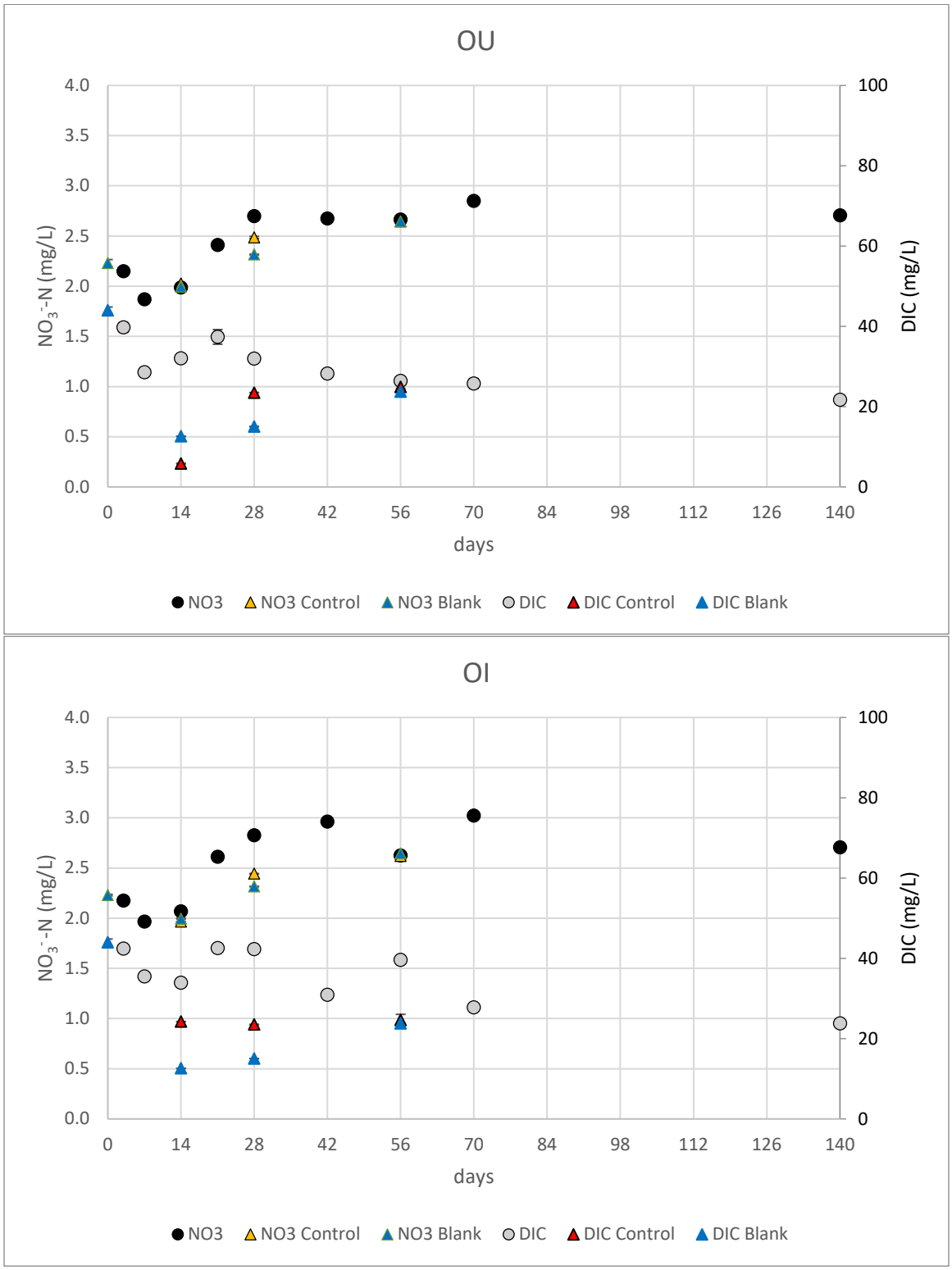


Figure 4-6: NO_3^- and DIC incubated in each closed experimental system

Black points denote nitrate (NO_3^-) concentration and grey points denote dissolved inorganic carbon (DIC). Triangles are control systems.

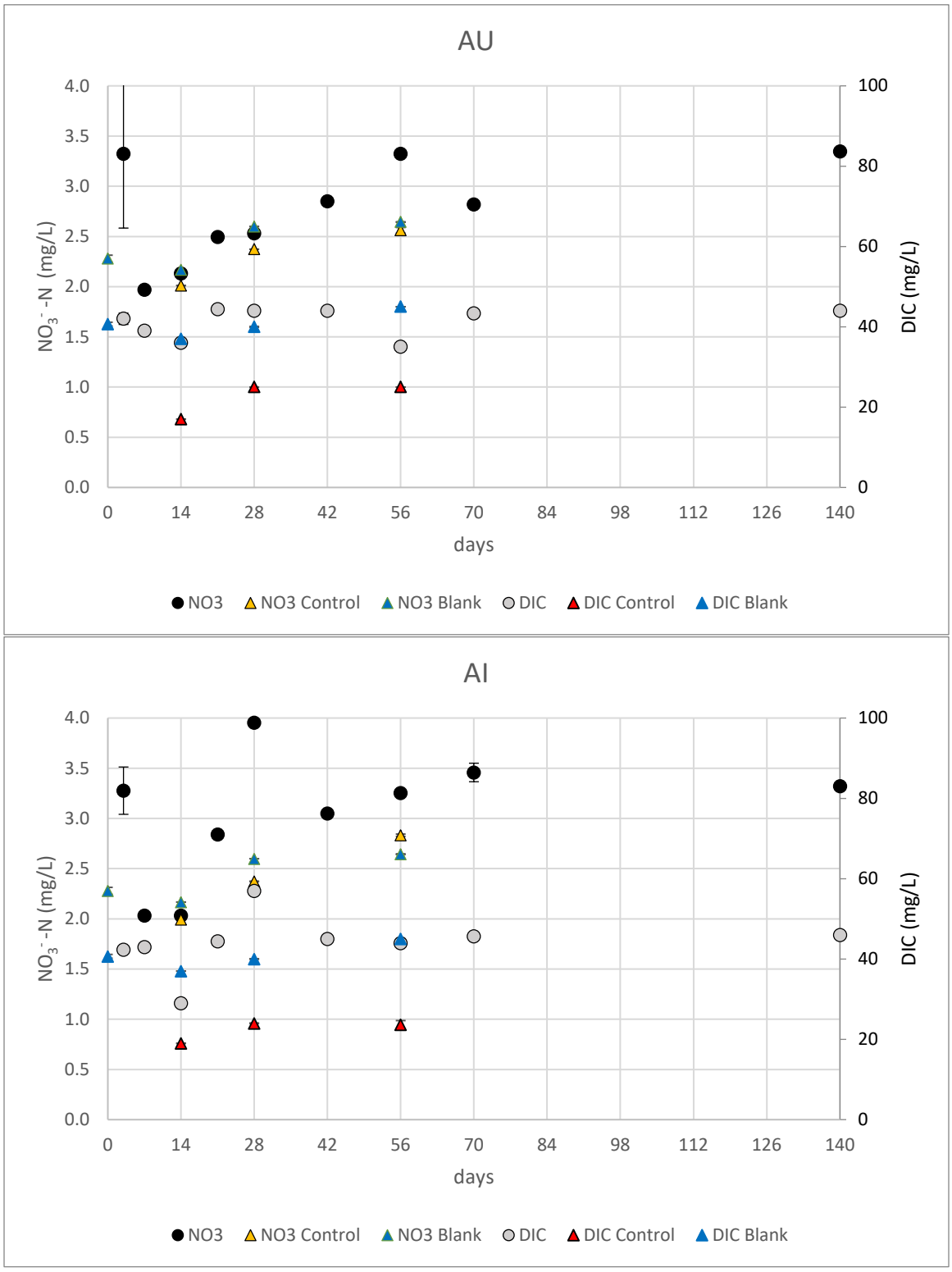


Figure 4-7: $\delta^{15}N$ of NO_3^- and sediment nitrogen incubated in open experimental systems

Grey points represent $\delta^{15}N$ of nitrate and black points are $\delta^{15}N$ of sediment nitrogen.

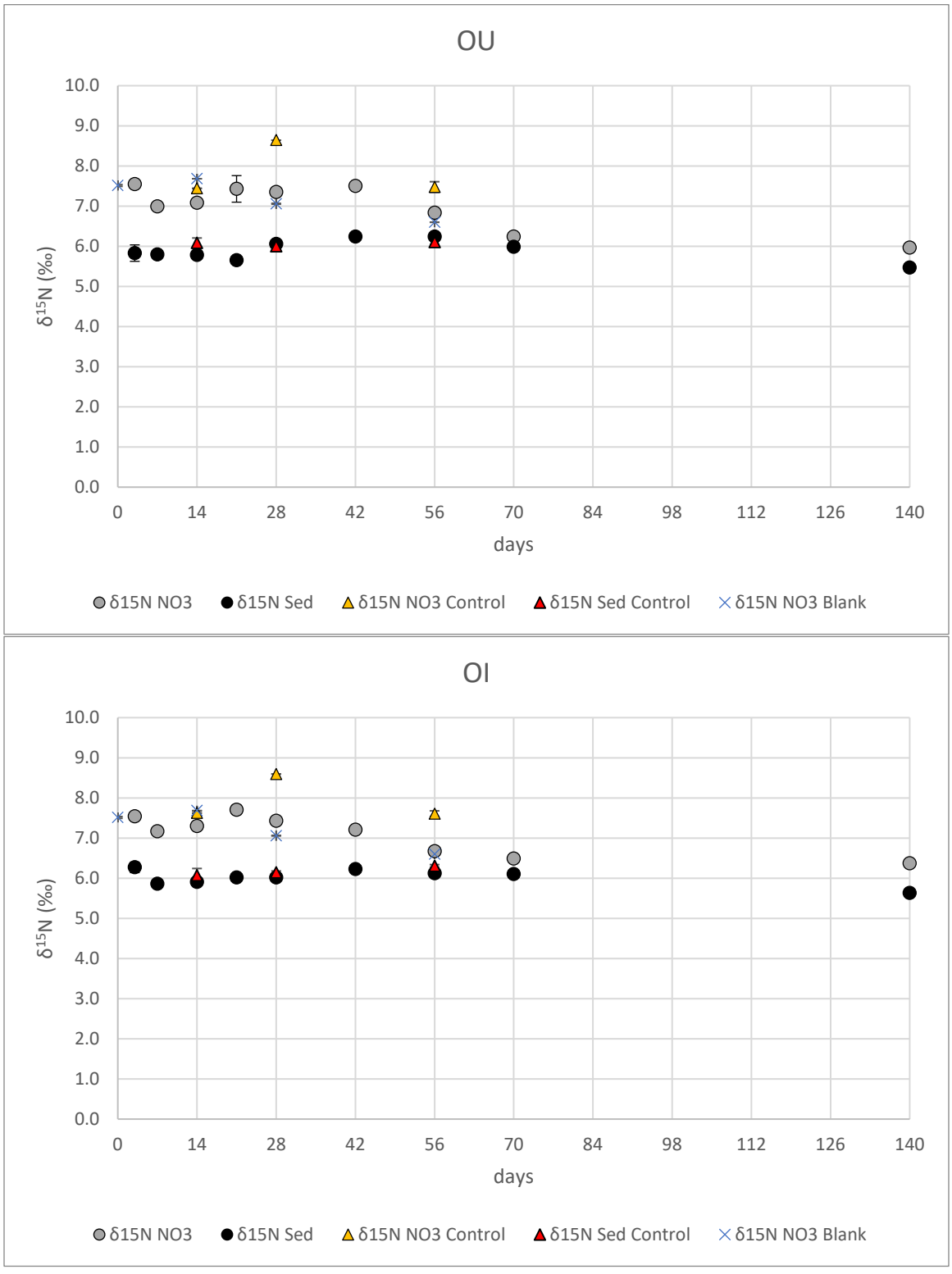


Figure 4-8: $\delta^{15}N$ of NO_3^- and sediment nitrogen incubated in closed experimental systems

Grey points represent $\delta^{15}N$ of nitrate and black points are $\delta^{15}N$ of sediment nitrogen.

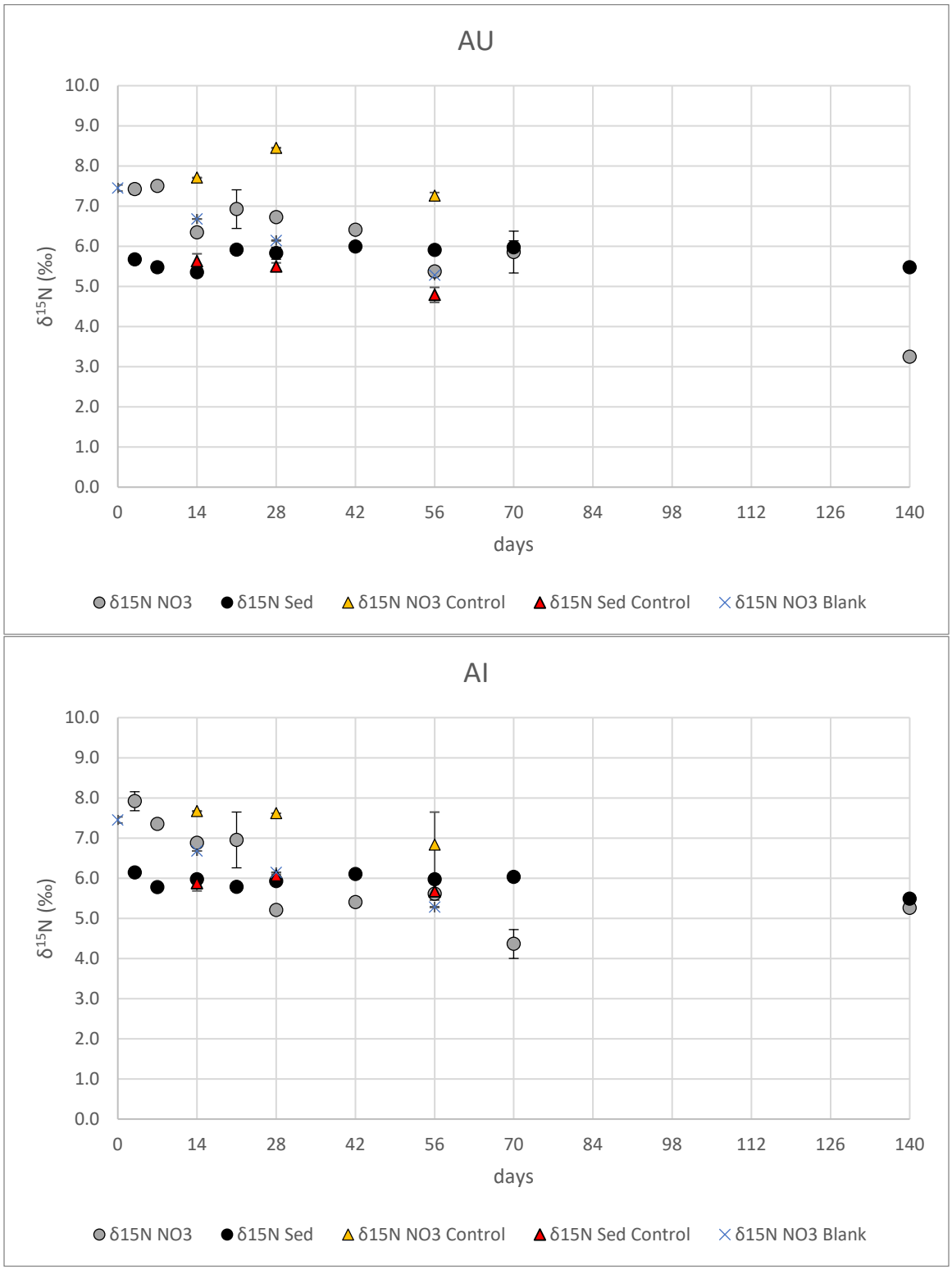


Figure 4-9: $\delta^{13}C$ of DIC and SOC incubated in each open experimental system

Grey points represent $\delta^{13}C$ of DIC and black points are $\delta^{13}C$ of sediment organic carbon.

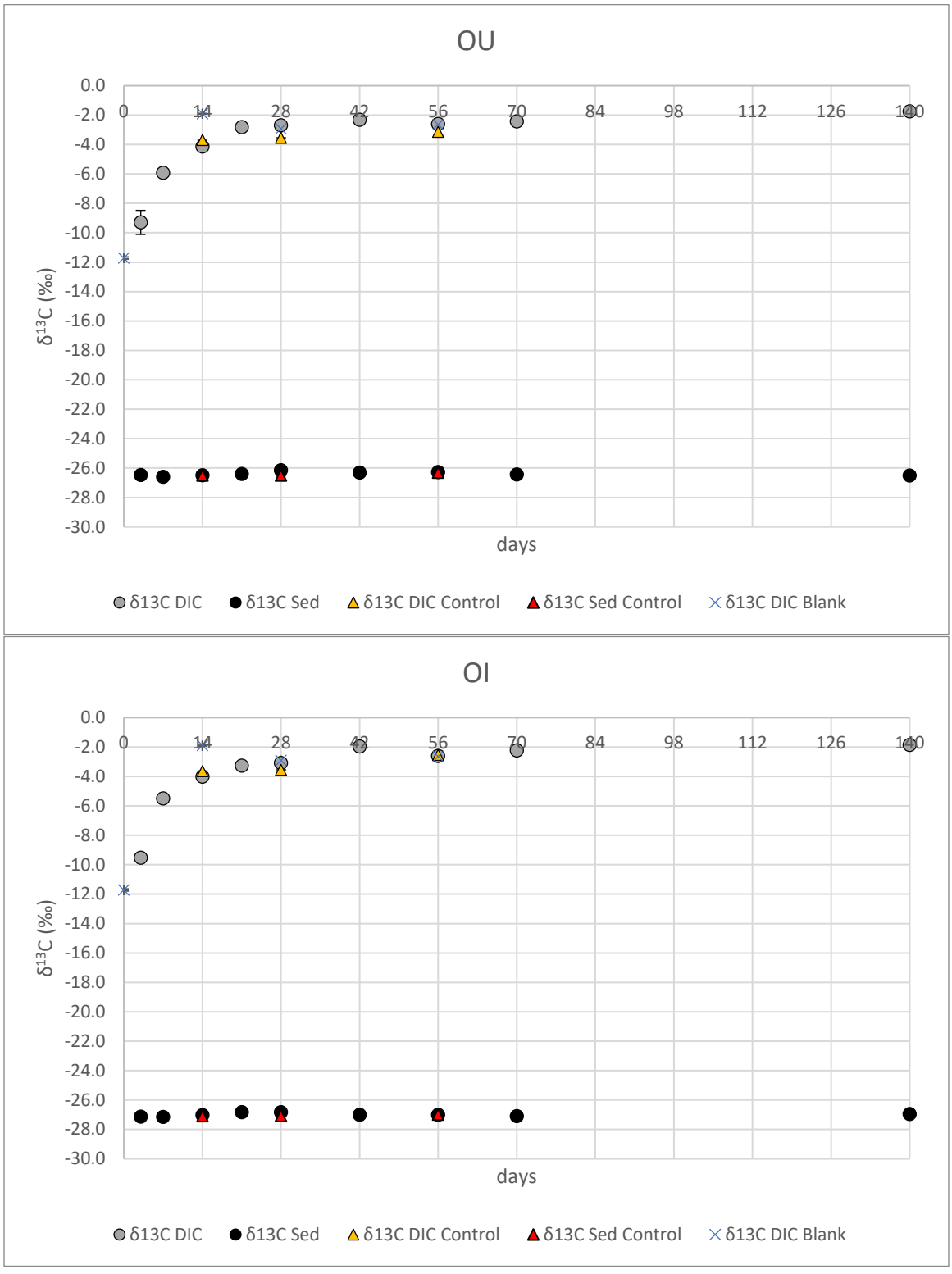


Figure 4-10: $\delta^{13}C$ of DIC and SOC incubated in each closed experimental system

Grey points represent $\delta^{13}C$ of DIC and black points are $\delta^{13}C$ of sediment organic carbon.

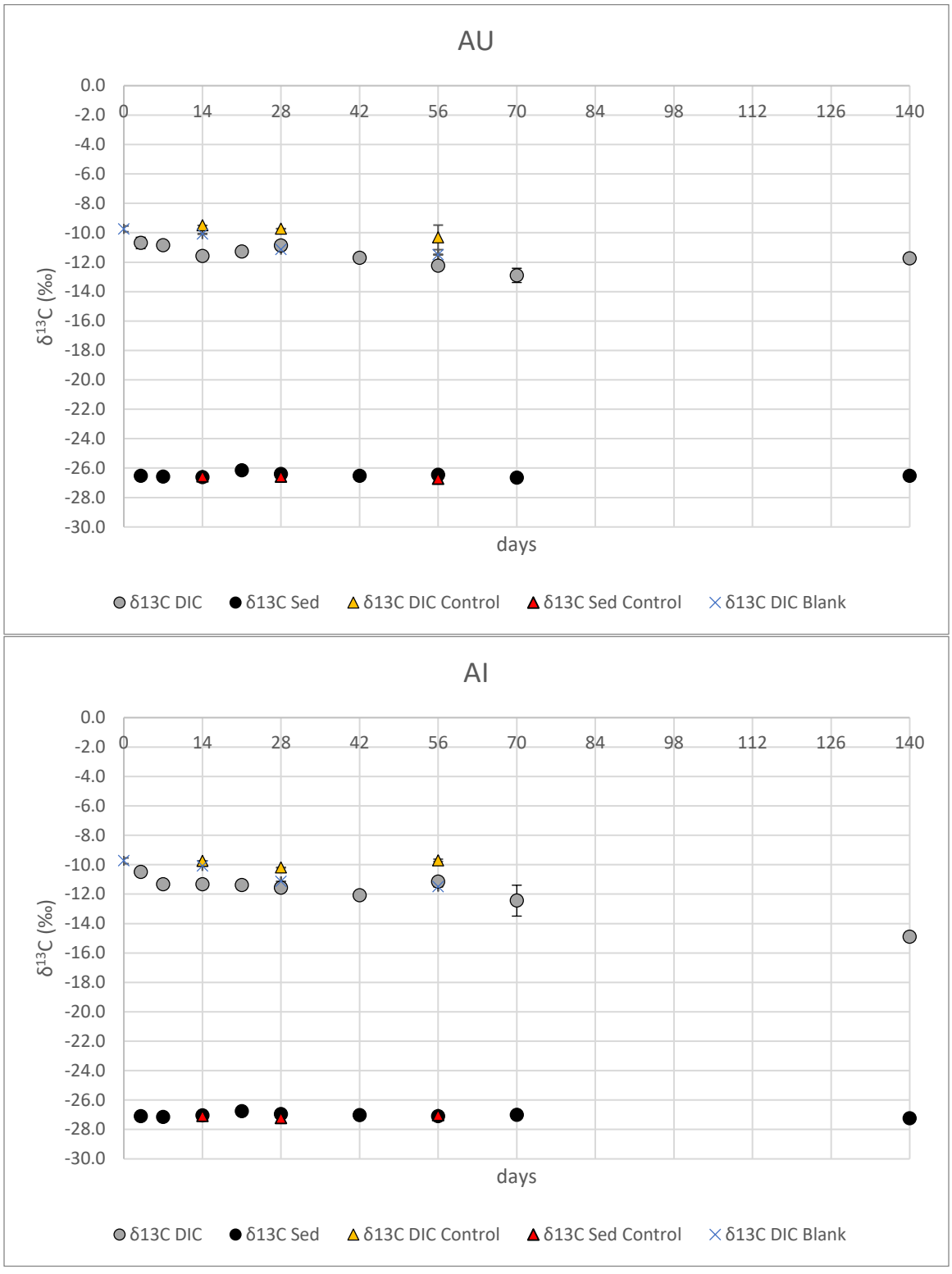
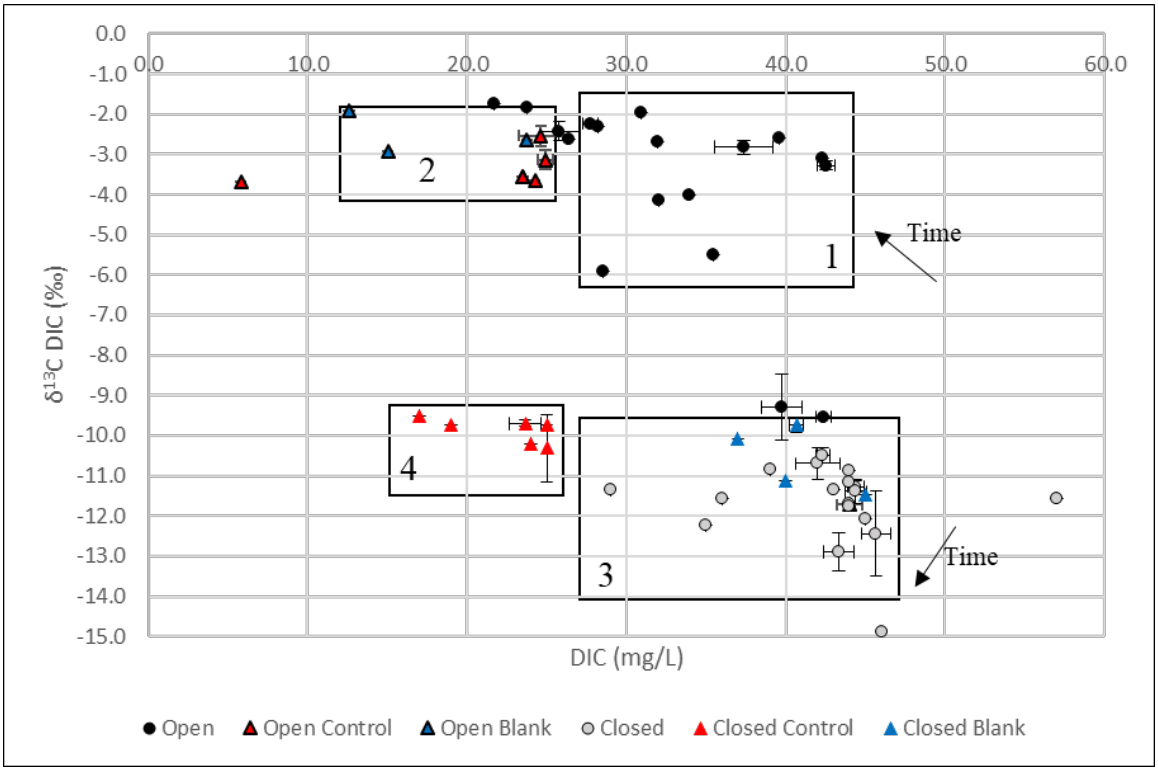


Figure 4-11: $\delta^{13}C$ of DIC and DIC (mg/L) incubated in each experimental system



Laboratory incubation study $\delta^{13}C$ DIC vs DIC (mg/L). The inorganic carbon species include carbon dioxide, carbonic acid, bicarbonate anion, and carbonate. The data is separated into 4 quadrants. 1) Full study with sediments (black points) incubated in an open system. $\delta^{13}C$ is approaching 0‰ over the 140 days. 2) Controls flasks in an open system. Sterilized systems have stable DIC concentrations. 3) Full study with sediments (grey points) incubated in a closed system. $\delta^{13}C$ is slightly decreasing with time. Blank controls included. 4) Sterilized controls in a closed system.

List of Time Series Figures from Incubation Experiments

Nitrogen Data

Figure 4-T1: Time series of Ammonia (NH_3-N) concentration (mg/L) throughout the incubation period.

Figure 4-T2: Time series of Dissolved Organic Nitrogen (DON-N) concentration (mg/L) throughout the incubation period.

Figure 4-T3: Time series of Nitrate (NO_3-N) concentration (mg/L) throughout the incubation period.

Figure 4-T4: Time series of $\delta^{15}N$ of Nitrate throughout the incubation period.

Figure 4-T5: Time series of $\delta^{18}O$ of Nitrate throughout the incubation period.

Figure 4-T6: Time series of Sediment Nitrogen (SN) mass (mg) throughout the incubation period.

Figure 4-T7: Time series of $\delta^{15}N$ of Sediment throughout the incubation period.

Carbon Data

Figure 4-T8: Time series of Dissolved Organic Carbon (DOC) concentration (mg/L) throughout the incubation period.

Figure 4-T9: Time series of Dissolved Inorganic Carbon (DIC) concentration (mg/L) throughout the incubation period.

Figure 4-T10: Time series of $\delta^{13}C$ of DIC throughout the incubation period.

Figure 4-T11: Time series of Sediment Organic Carbon (SOC) mass (mg) throughout the incubation period.

Figure 4-T12: Time series of $\delta^{13}C$ of Sediment throughout the incubation period.

Figure 4-T13: Time series of C/N ratio of dissolved and sediment phases throughout the incubation period.

Figure 4-T14: Time series of C/N ratio sediment throughout the incubation period.

Experimental Data

Figure 4-T15: Time series of Temperature (°C) throughout the incubation period.

Figure 4-T16: Time series of pH throughout the incubation period.

Figure 4-T1: Time series of Ammonia (NH_3-N) concentration (mg/L) throughout the incubation period.

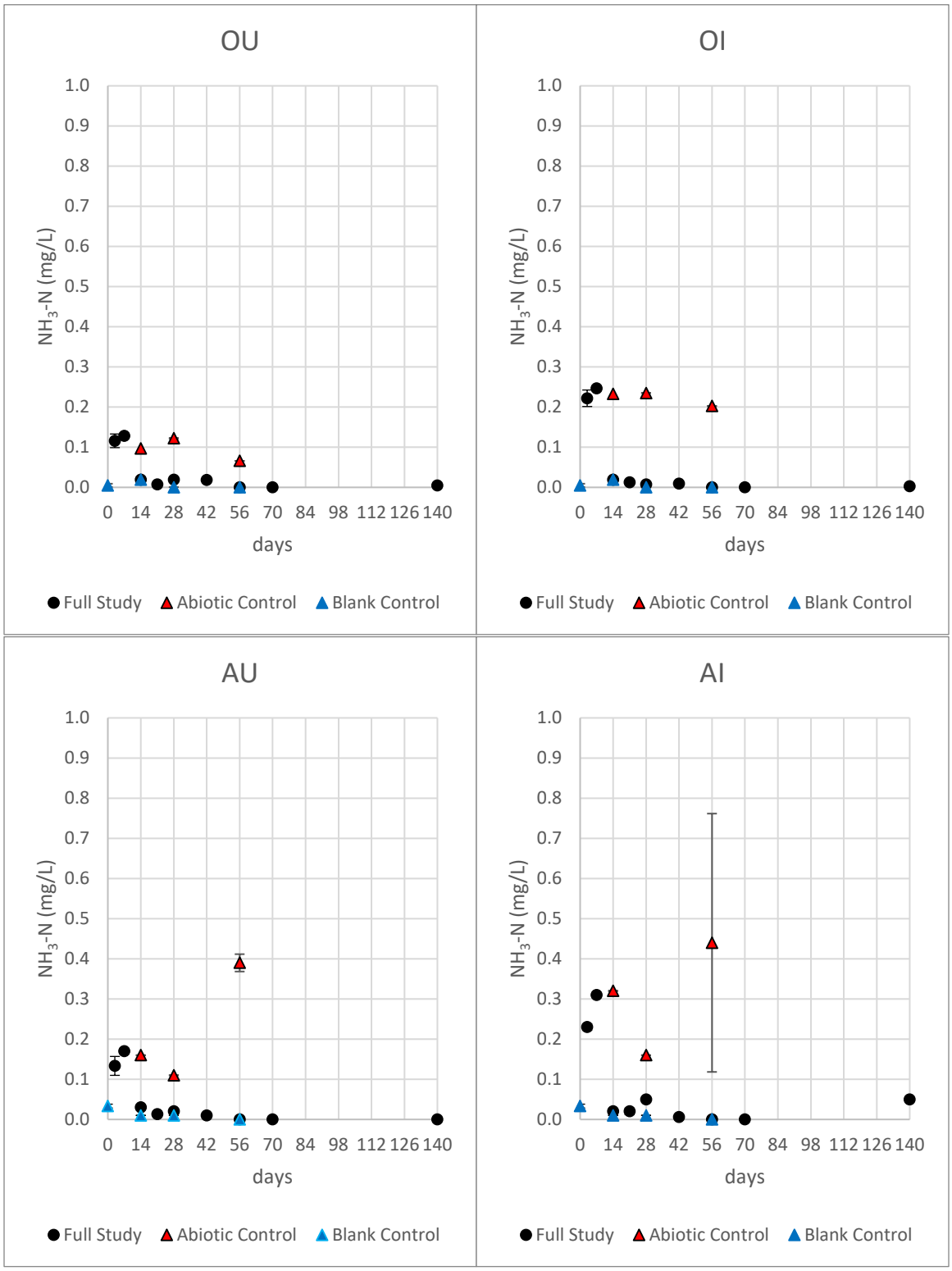


Figure 4-T2: Time series of Dissolved Organic Nitrogen (DON-N) concentration (mg/L) throughout the incubation period.

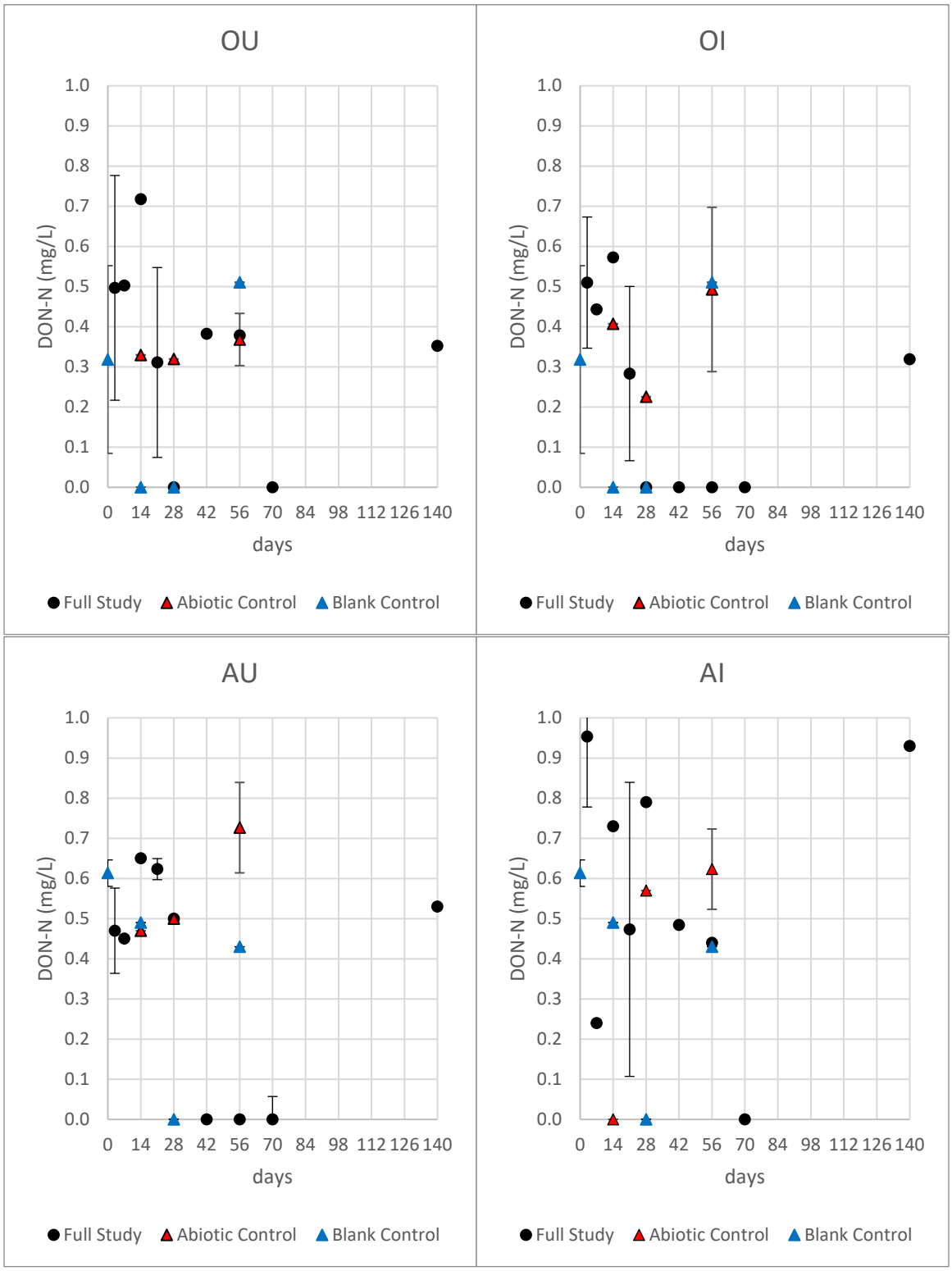


Figure 4-T3: Time series of Nitrate (NO_3-N) concentration (mg/L) throughout the incubation period.

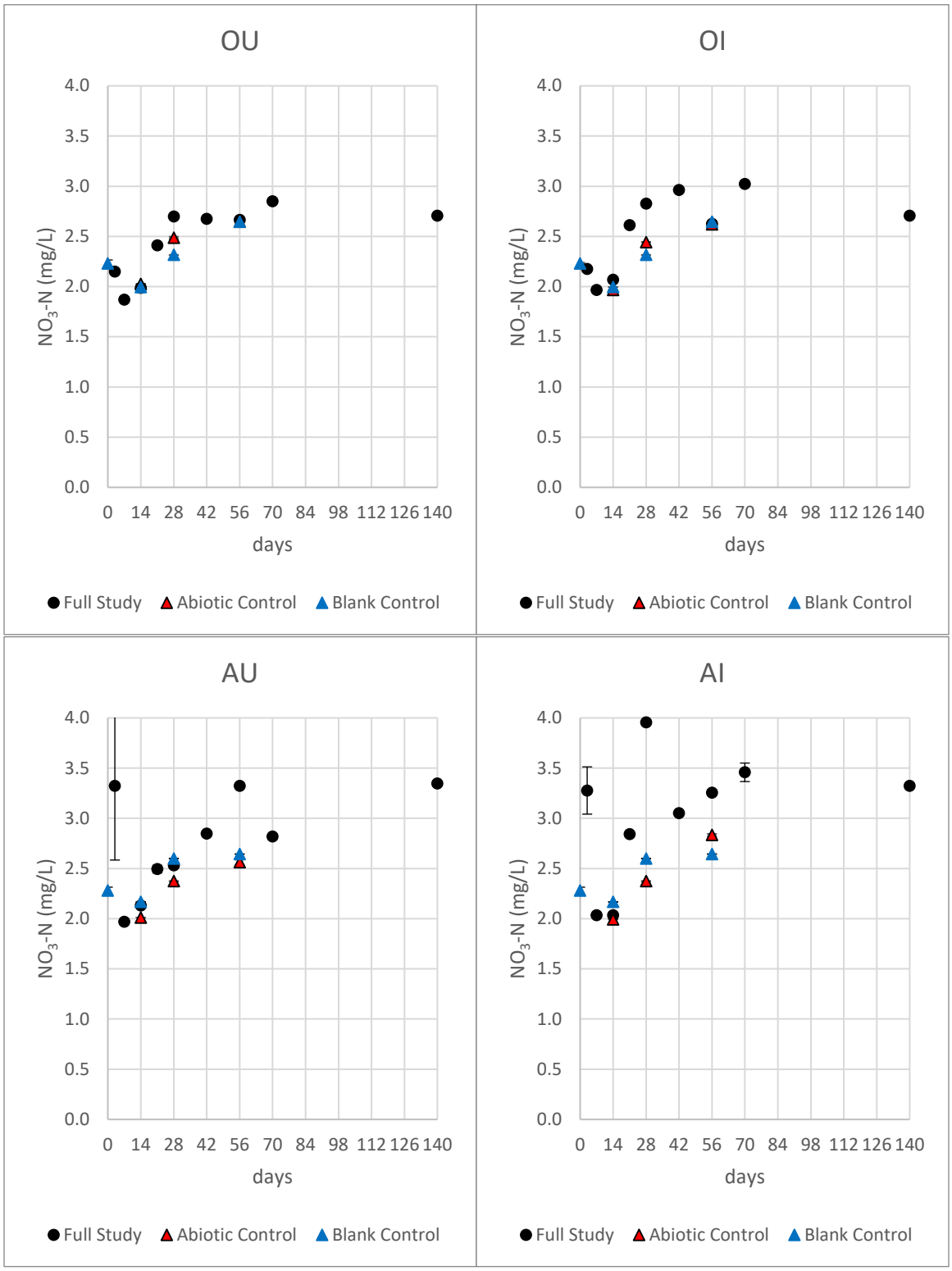


Figure 4-T4: Time series of $\delta^{15}N$ of Nitrate throughout the incubation period.

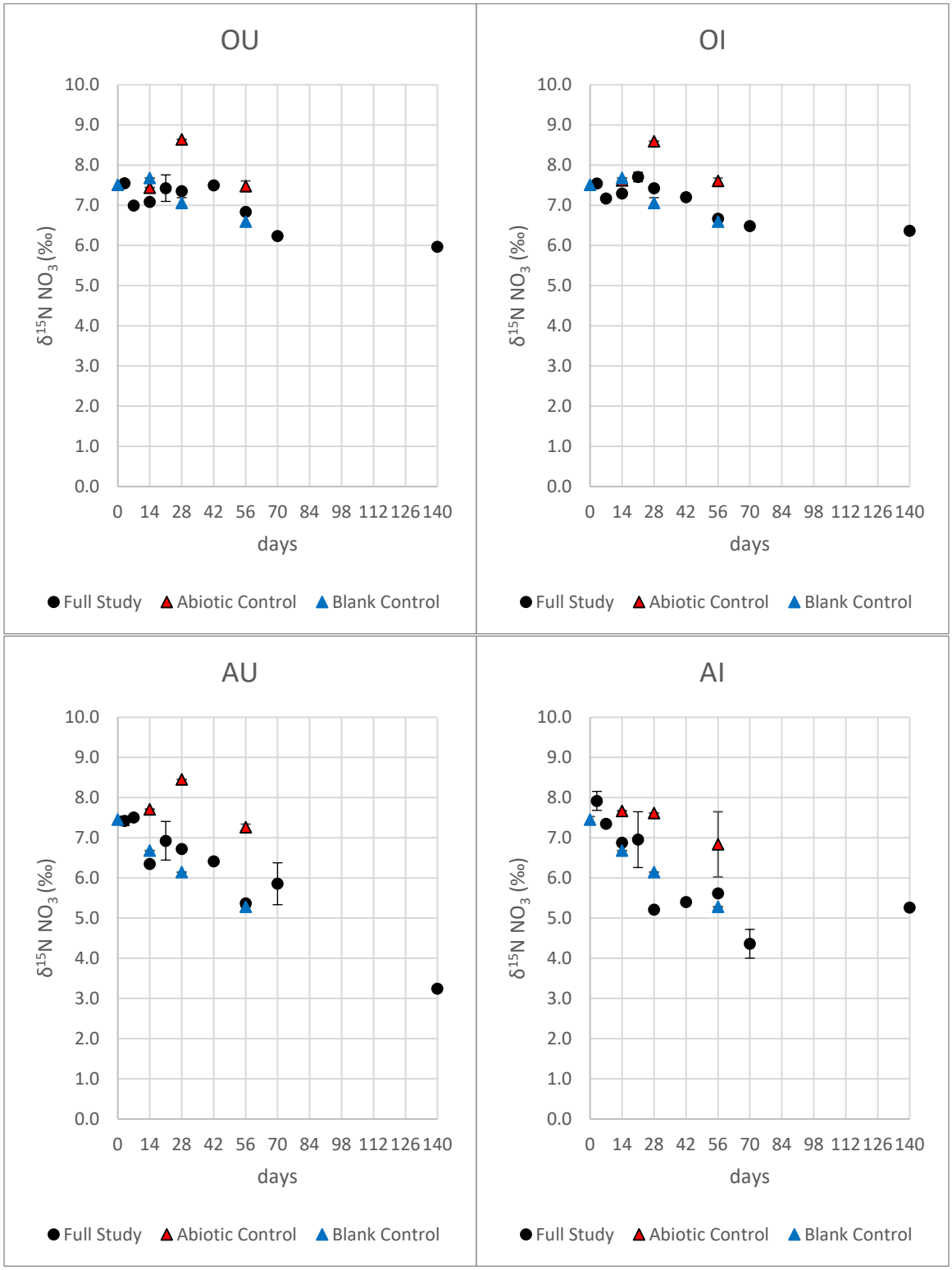


Figure 4-T5: Time series of $\delta^{18}O$ of Nitrate throughout the incubation period.

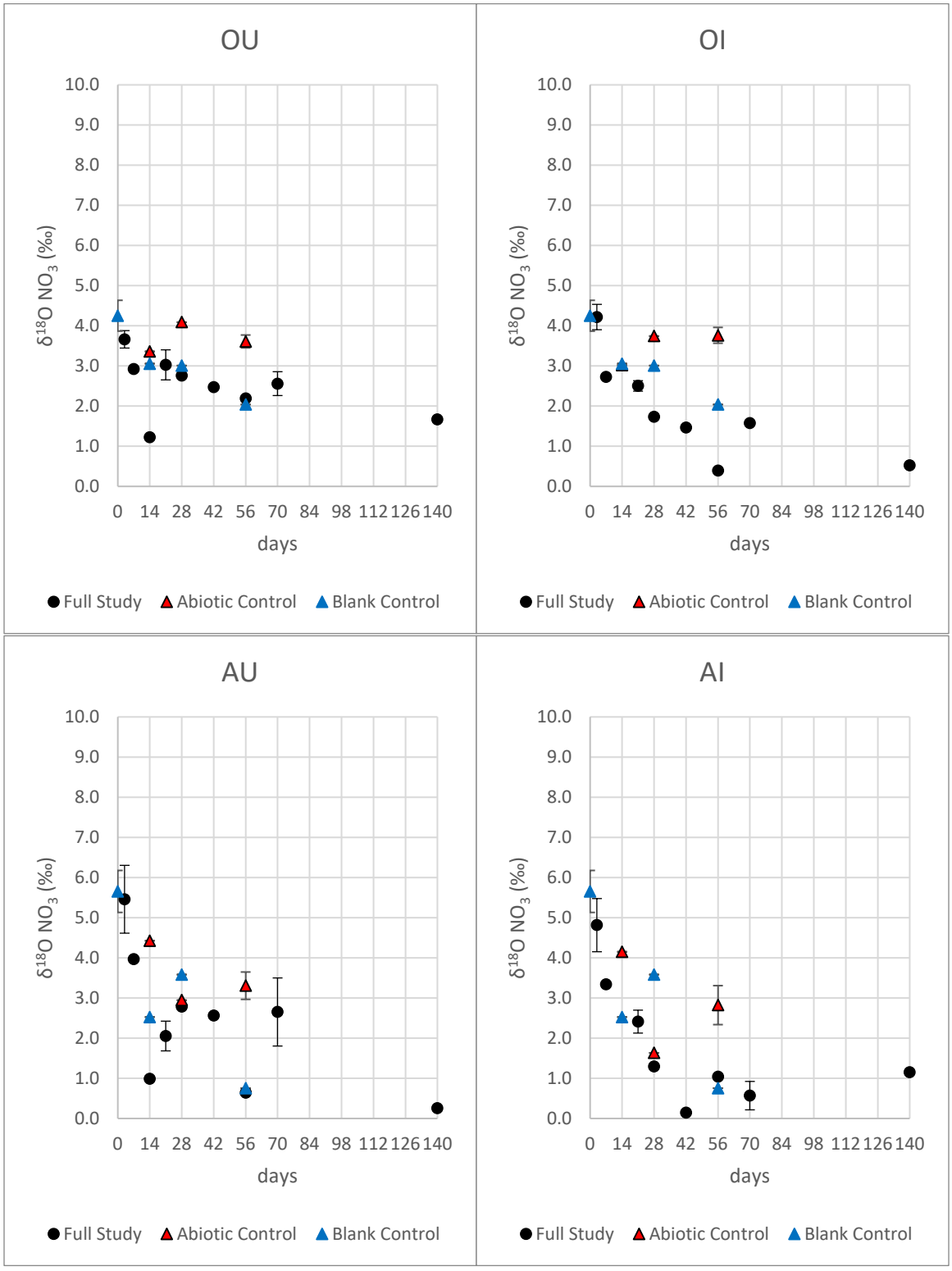


Figure 4-T6: Time series of Sediment Nitrogen (SN) mass (mg) throughout the incubation period.

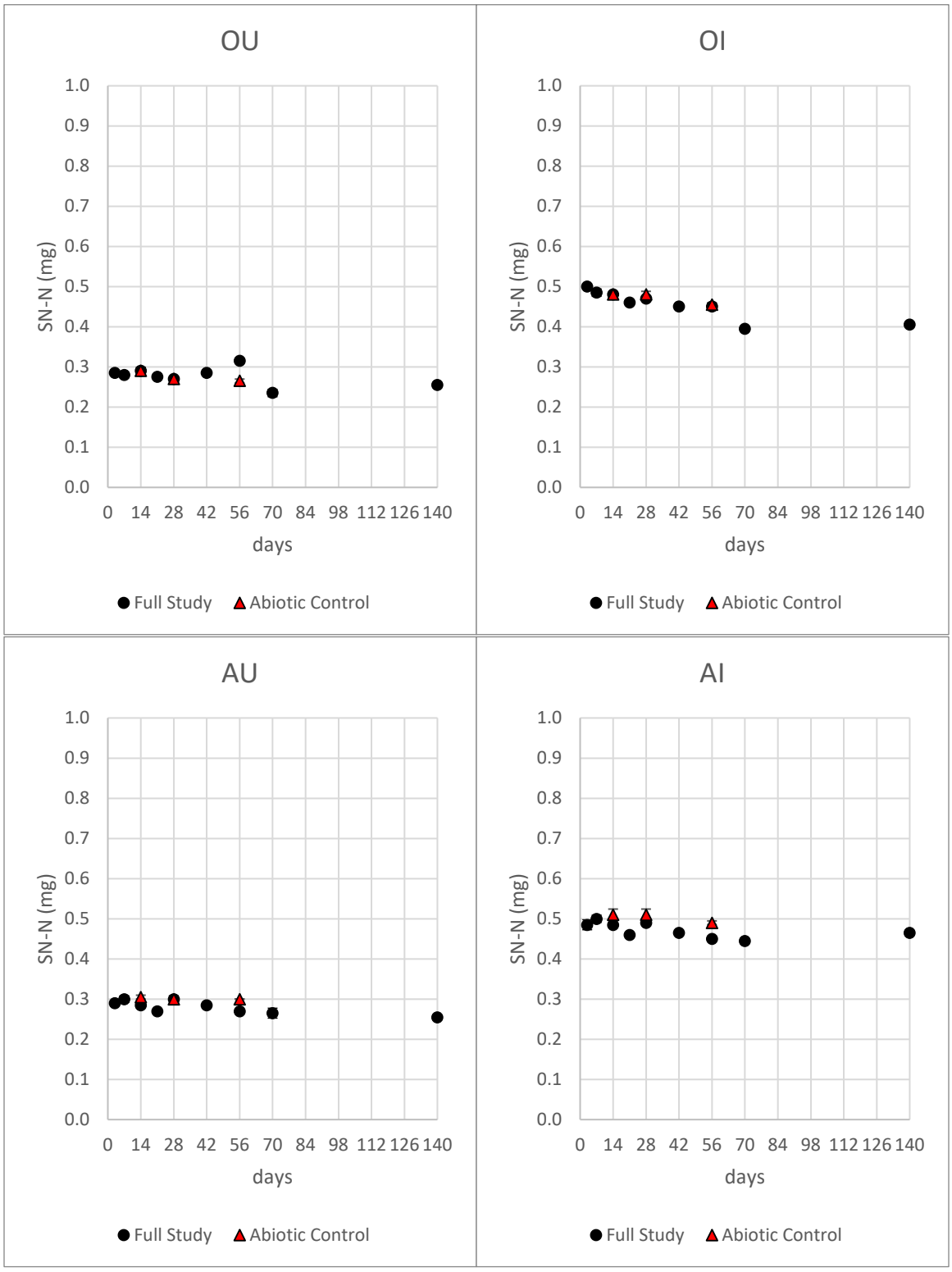


Figure 4-T7: Time series of $\delta^{15}N$ of Sediment throughout the incubation period.

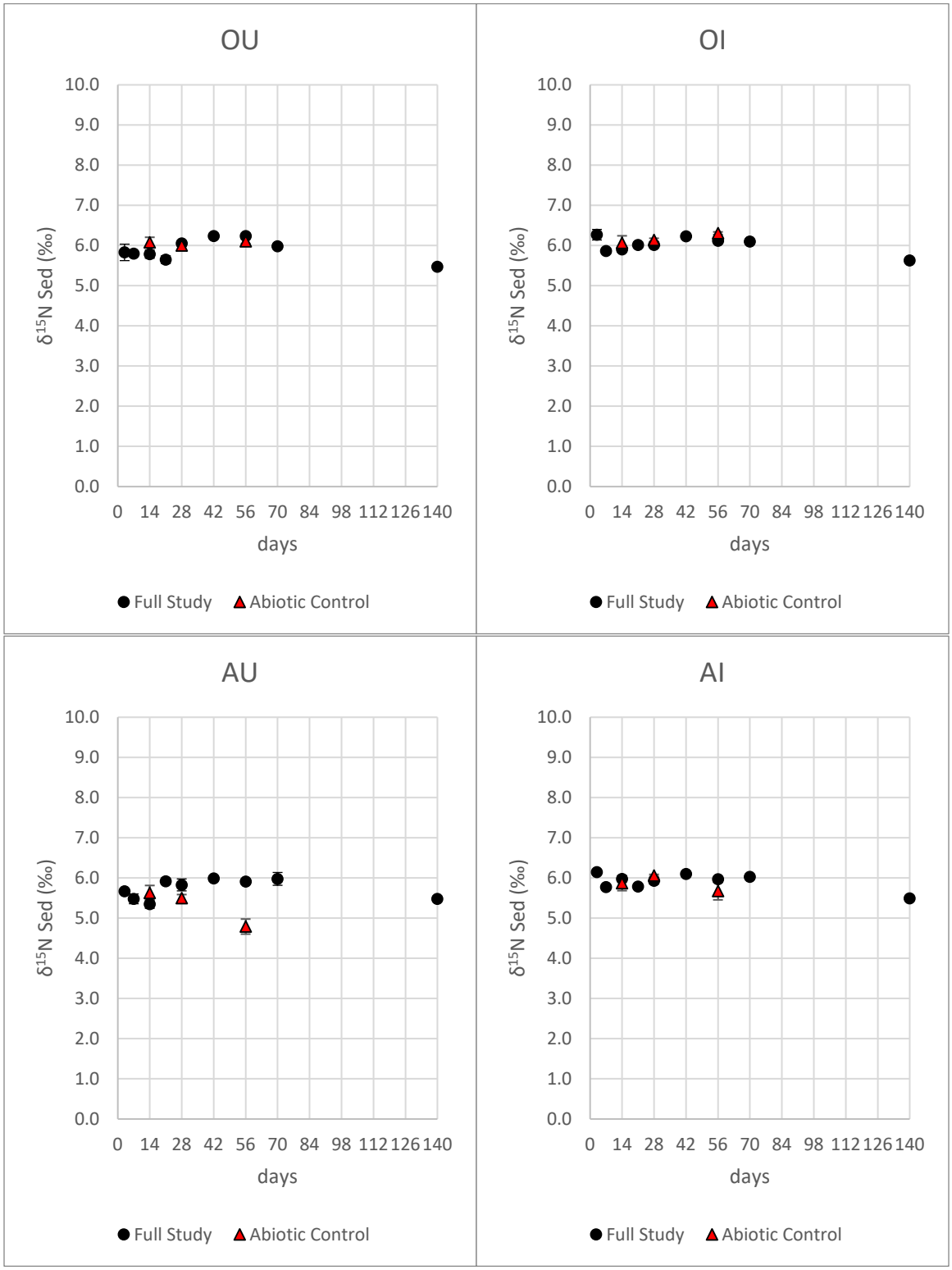


Figure 4-T8: Time series of Dissolved Organic Carbon (DOC) concentration (mg/L) throughout the incubation period.

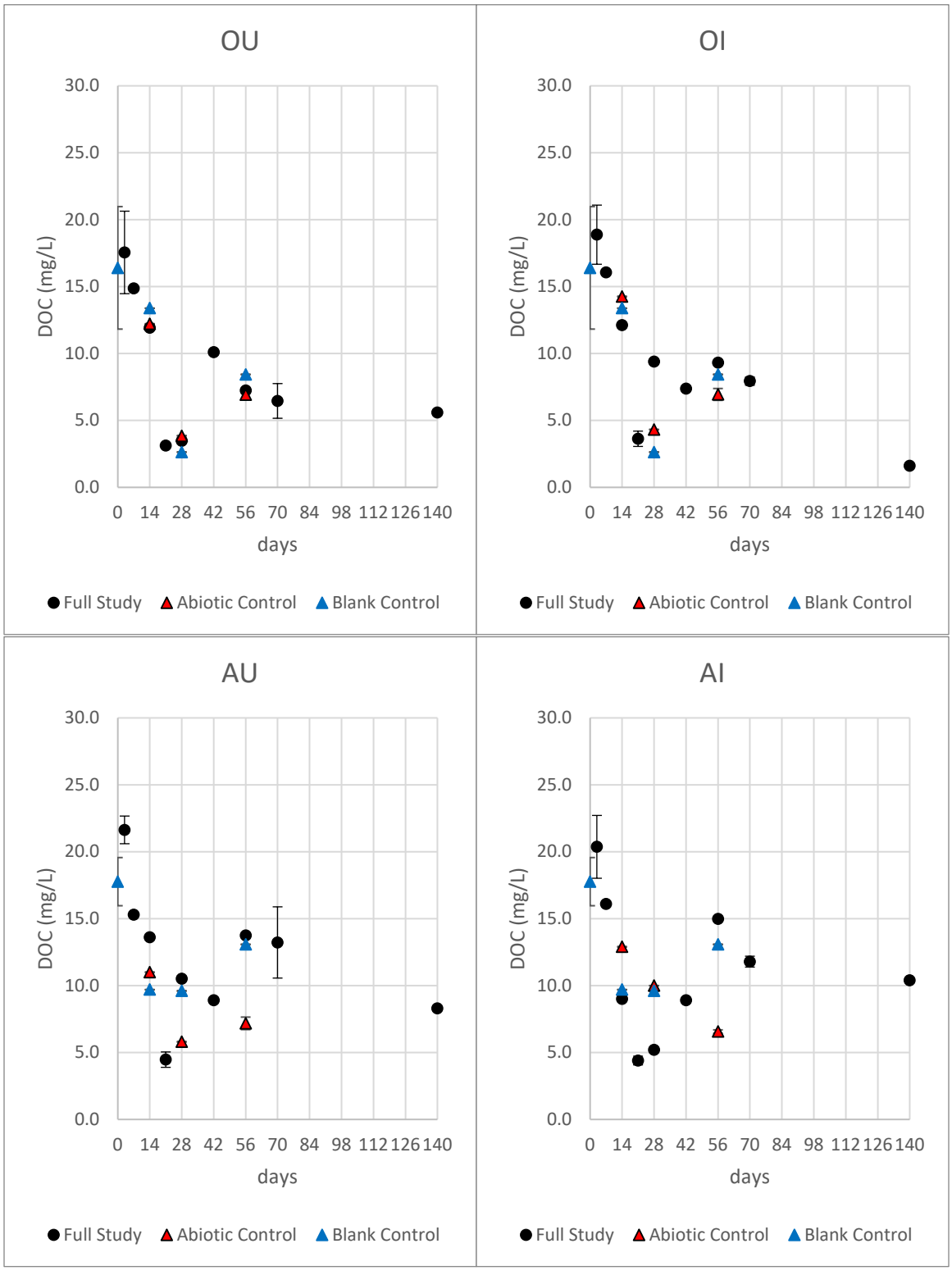


Figure 4-T9: Time series of Dissolved Inorganic Carbon (DIC) concentration (mg/L) throughout the incubation period.

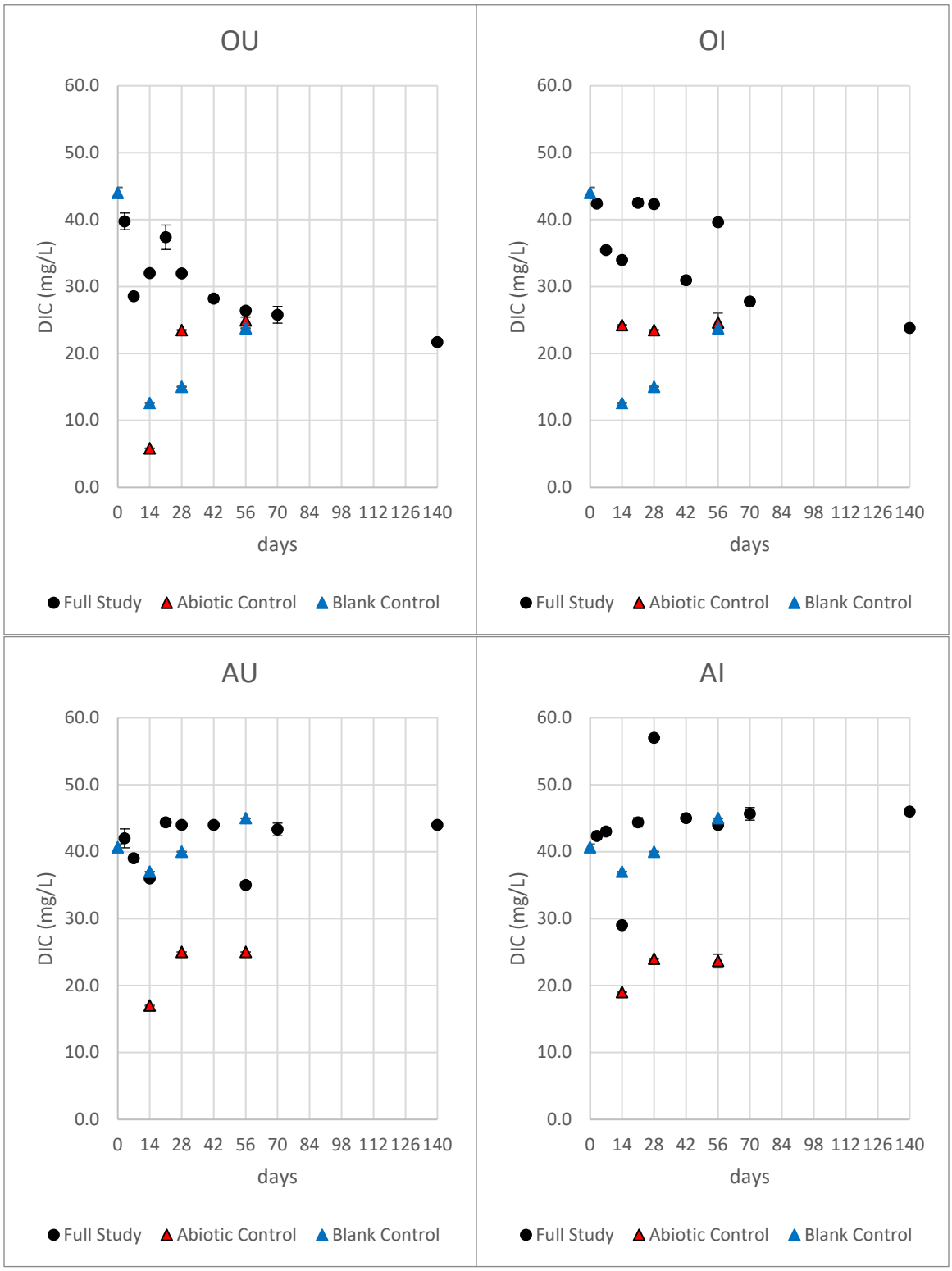


Figure 4-T10: Time series of $\delta^{13}C$ of DIC throughout the incubation period.

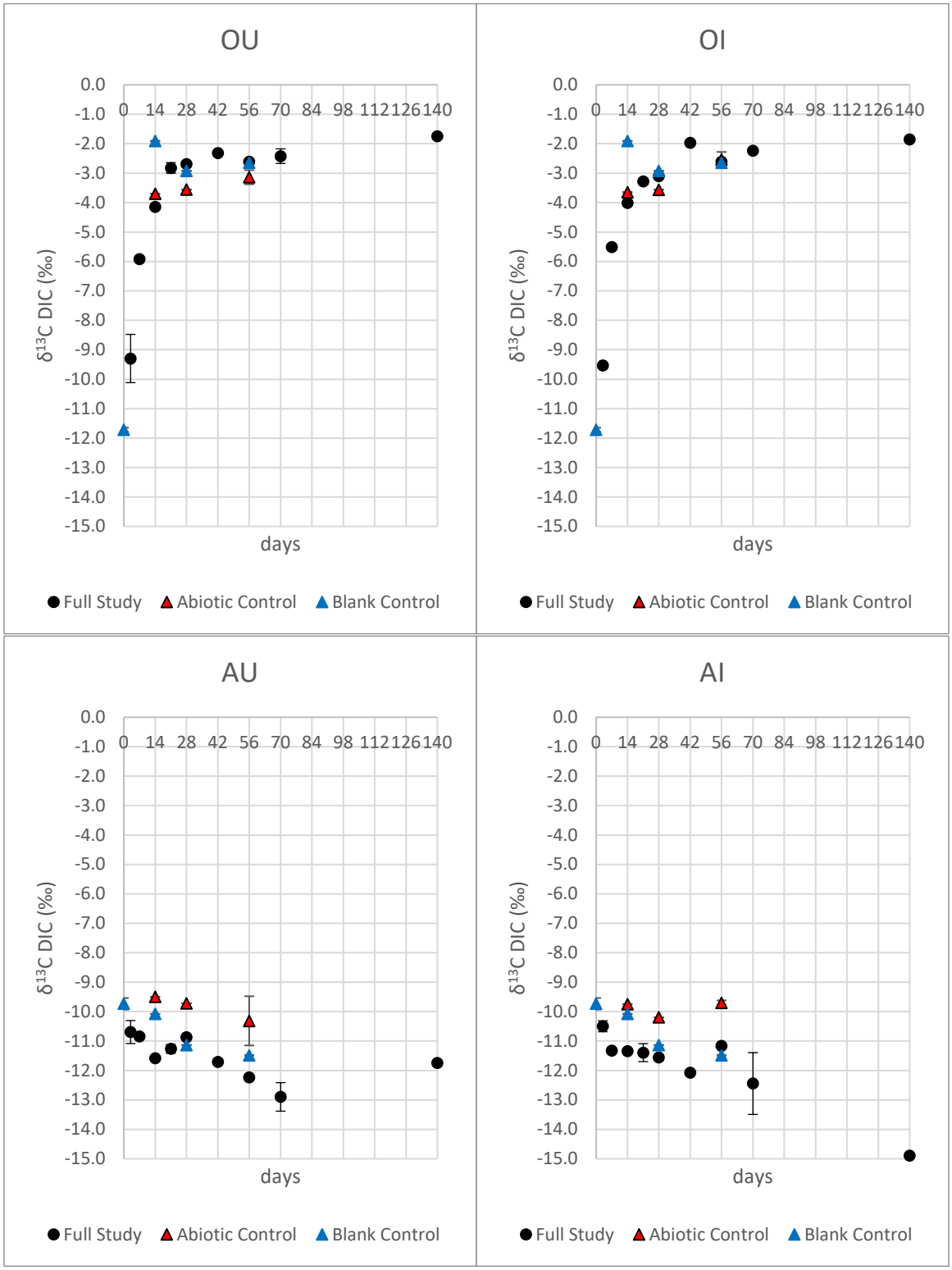


Figure 4-T11: Time series of Sediment Organic Carbon (SOC) mass (mg) throughout the incubation period.

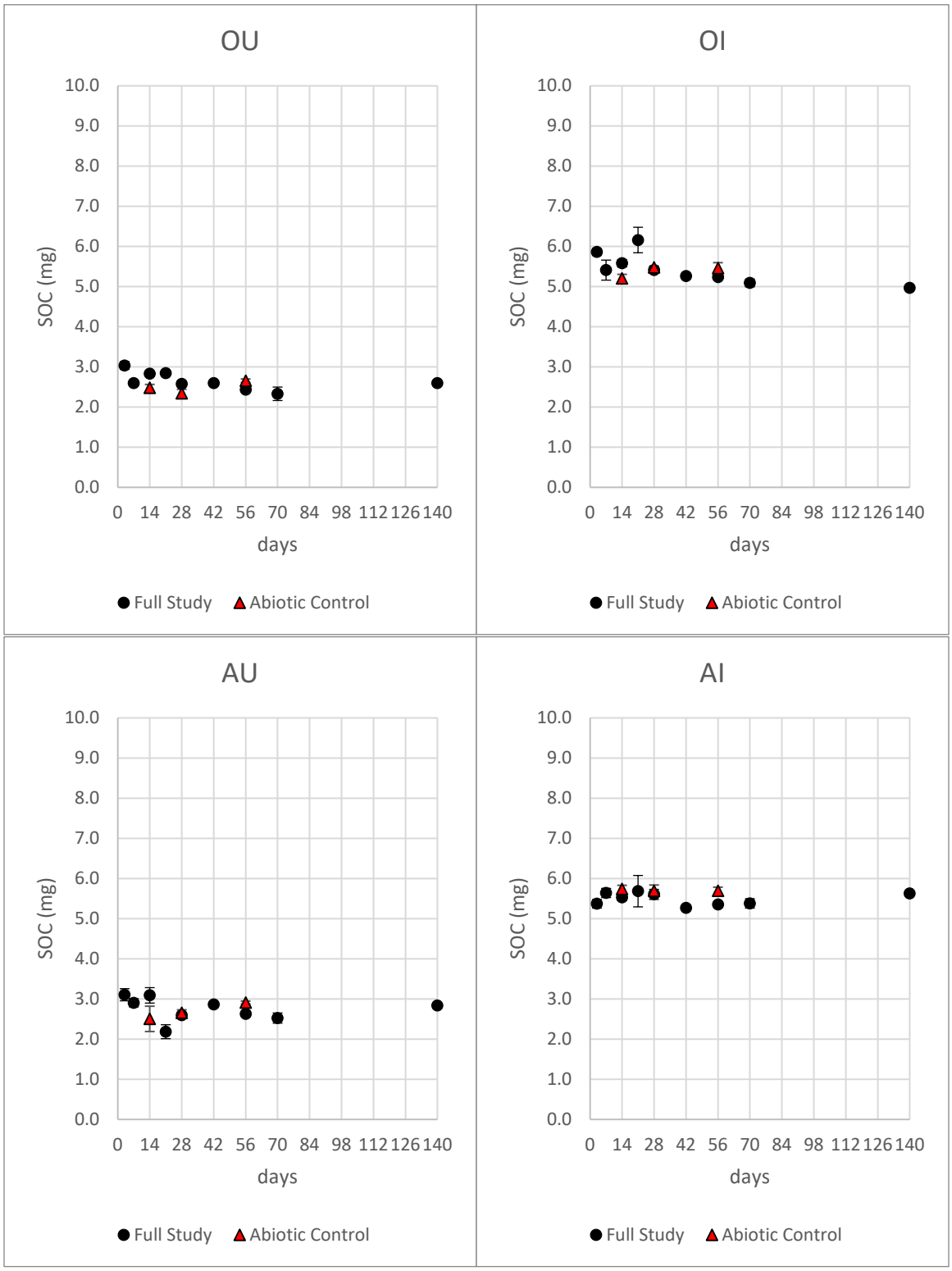


Figure 4-T12: Time series of $\delta^{13}C$ of Sediment throughout the incubation period.

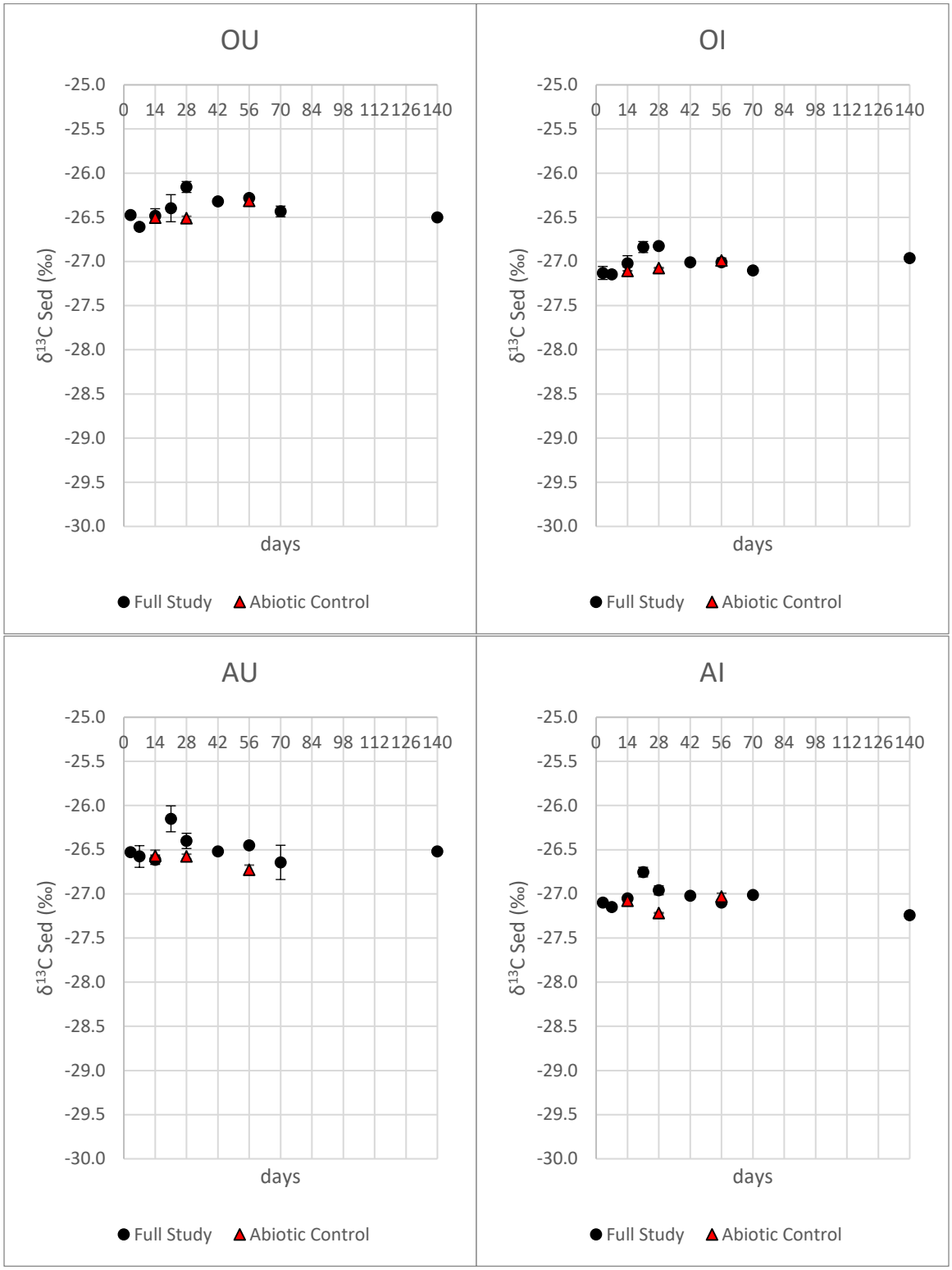


Figure 4-T13: Time series of C/N ratio of dissolved organic matter (DOC/DON) throughout the incubation period.

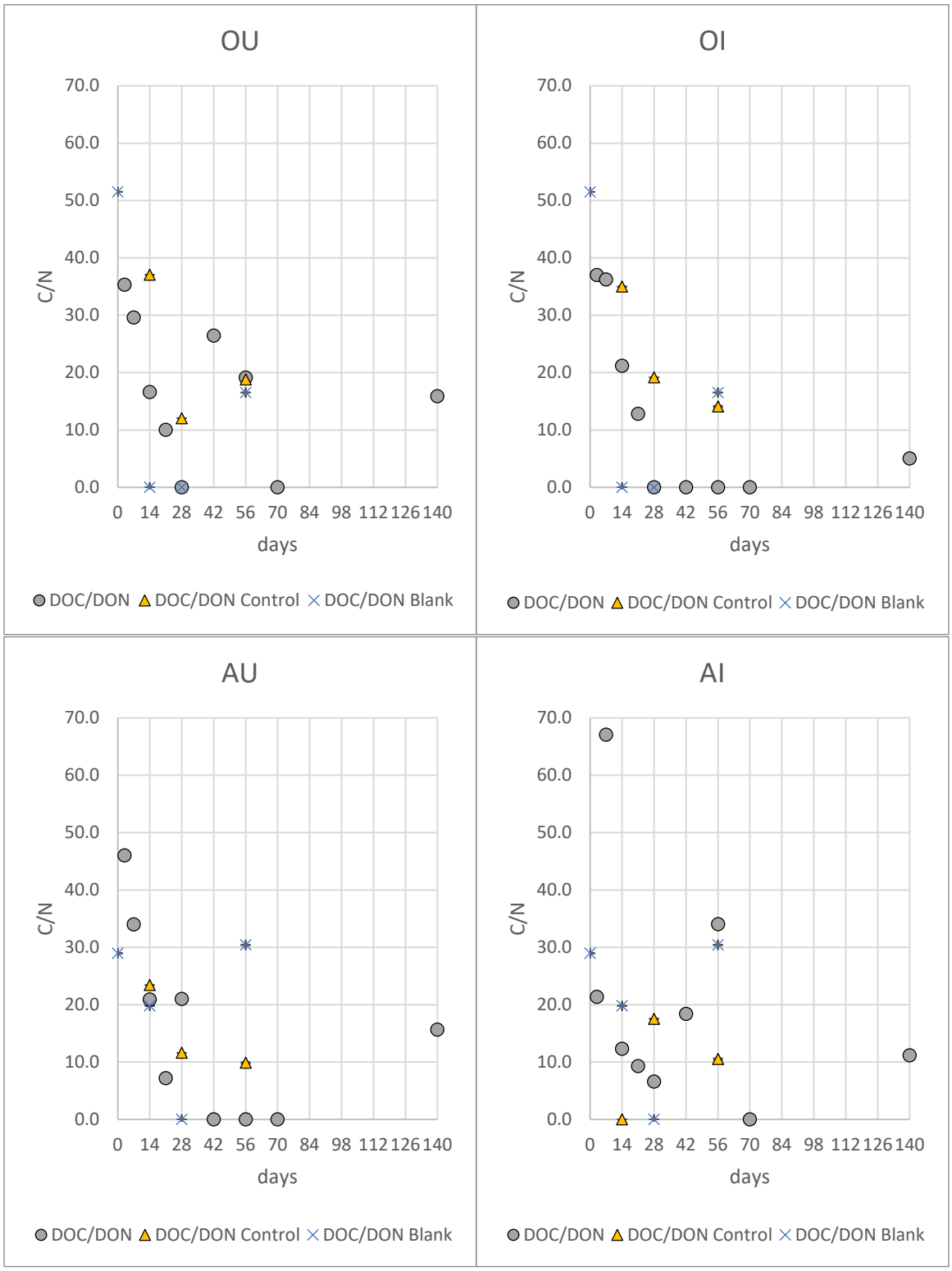


Figure 4-T14: Time series of C/N ratio sediment throughout the incubation period.

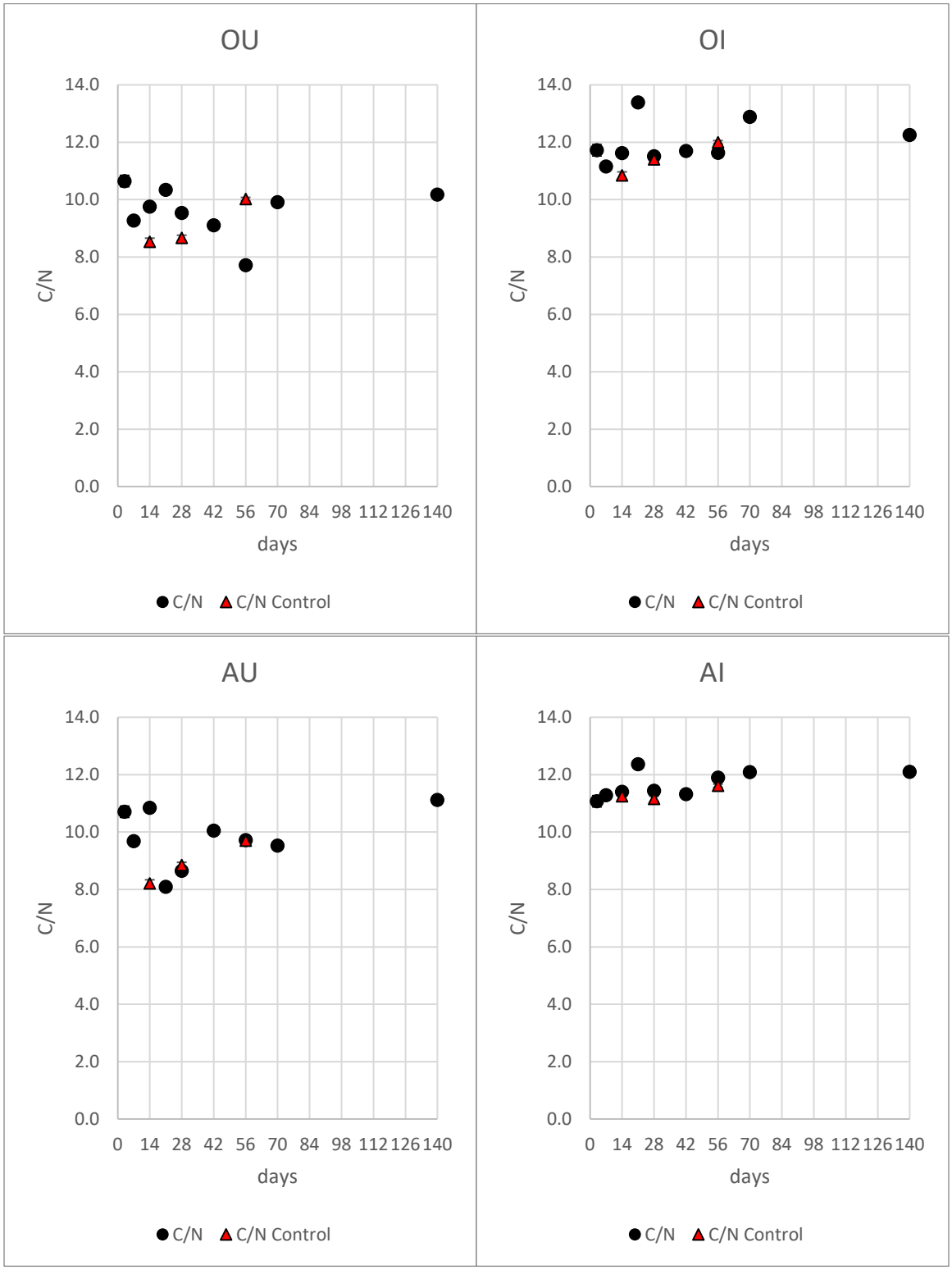


Figure 4-T15: Time series of Temperature (°C) throughout the incubation period.

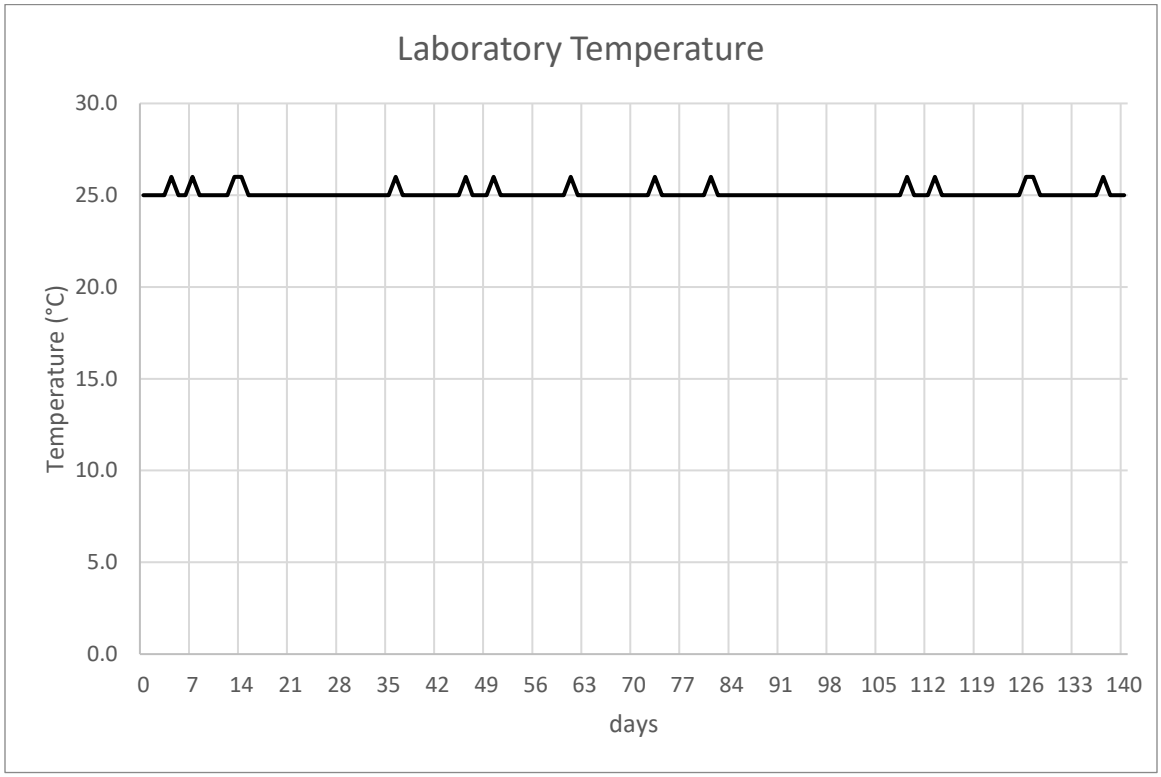
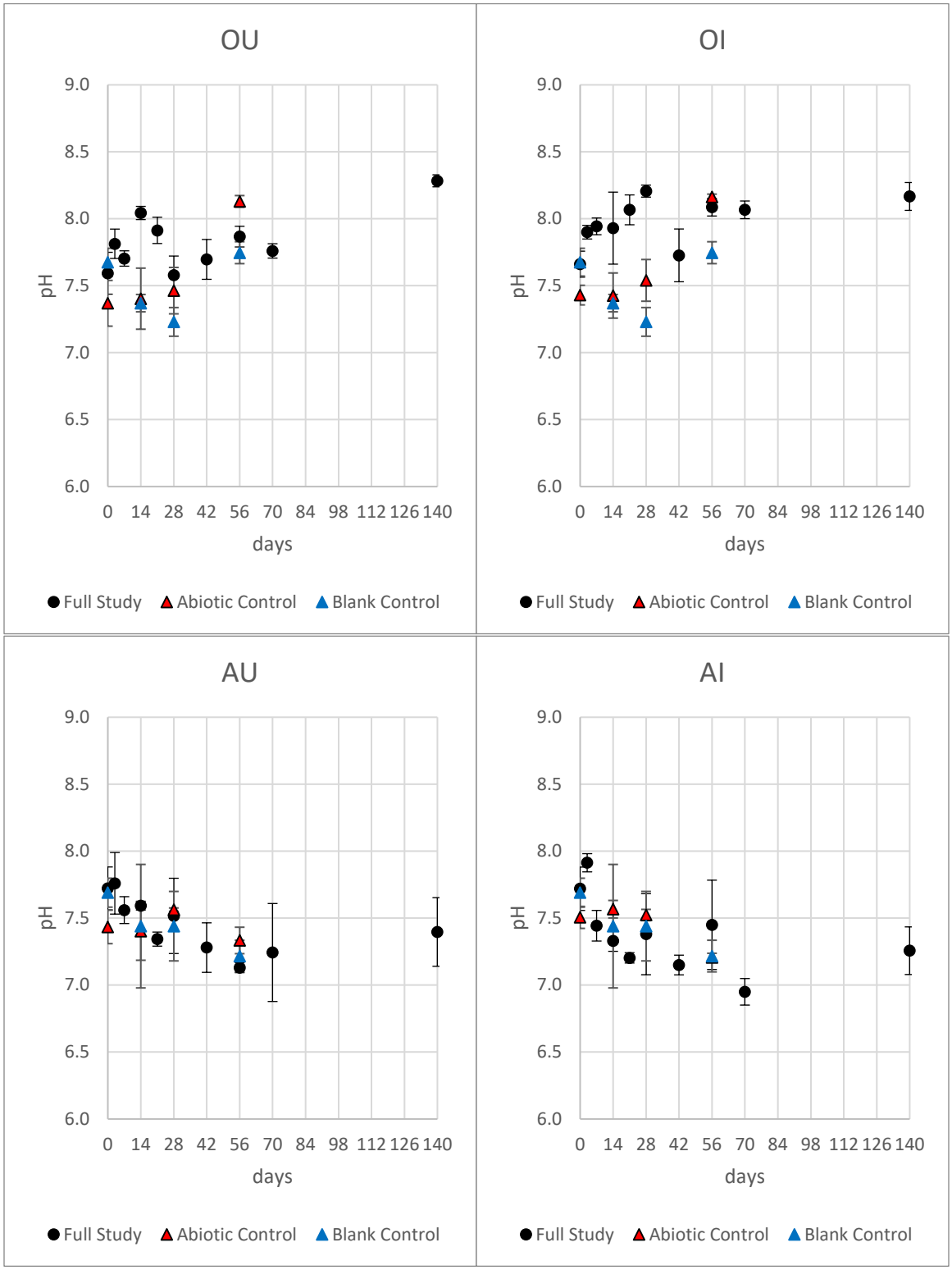


Figure 4-T16: Time series of pH throughout the incubation period.



Chapter 5 - Modelling Results, Field Data Results and Discussion

The methods and data results from the incubation study allowed us to make some further assumptions and considerations regarding performance of the modelling for the incubation study, which are discussed in sections 5.1 through 5.4. Key results and interpretation from the data results that were carried forward from the methods to the modelling results can be explained as follows.

First, we consider the potential for two sediment pools and two dissolved organic matter pools. We consider this in modelling due to our methods for collecting the sample types, our understanding of stream systems, and our knowledge from the C and N modelling literature (Alvarez and Alvarez, 2000; Benbi and Richter, 2002).

Second, we consider the dominant reactions occurring to be carbon oxidation, CO_2 evasion, nitrogen mineralization, and nitrification in the open flasks and carbon oxidation, nitrogen mineralization, and nitrification in the closed flasks. These reactions are expected to be occurring based on our data results and our understanding of these types of systems from the literature.

Third, we only carry our modelling and their fitting of the mentioned processes to day 70. We do not consider data from day 140. The reason is, we believe additional processes may be occurring between days 70 and 140, however we do not have sufficient data to provide evidence of such processes. For example, some microbial growth may be occurring during this time, which is partially evidenced by the very high DON numbers. It is also possible that denitrification was occurring during this time period from day 70

to 140. It is not clear, but dissolved oxygen conditions in sediment accrued on the bottom of the flasks could occur.

Fourth, we start modelling of some constituents on day 3 because our modelling does not physically account for hydrolysis that may have occurred.

Fifth, we do not account for any abiotic processes occurring in our modelling. Abiotic processes could potentially occur in the study, however, our sterilized abiotic controls did not work properly and therefore only biotic processes are considered.

Sixth, we assume first order decay functions discretized at a daily time step is sufficient for the reactions occurring in the flasks. First order kinetics refers to chemical reactions whose rate of reaction depends on the concentration of the reactant. The first order rate law is assumed because it considers both the rate constant and reactant concentration. Some research has proposed zero order functions may be more appropriate for some reactions (e.g., soil N mineralization, Benbi and Richter, 2002), and will investigate such models as another potential solution in future work.

Seventh, we decided to avoid explicitly modelling evasion in the modelling section. There are a number of papers who model evasion in stream environments using rate equations based on partial pressure of carbon dioxide in water and the atmosphere (e.g., Wallin et al., 2013 and references therein), however, we found this relationship to not match well with our laboratory incubation data. We may investigate other methods for evasion modelling in future work.

Eighth, in our modelling, our Rayleigh relationships are assumed appropriate and discretized on daily time steps. The fraction remaining is updated daily, and this method

may in fact overestimate and linearize the solution. We may consider the sensitivity of other numerical approximations and parameterizations in future work.

Statistical evidence in the form of depleted NH_4^+ and increasing NO_3^- in all experiments suggests nitrification is a relevant and occurring process whereas denitrification is not expected to be occurring. This is as expected as the amount of dissolved oxygen (DO= 6.53 ± 1.03 mg l^{-1}) within flasks support aerobes like nitrifyers and is detrimental to the proliferation of strict anaerobes such as denitrifyers. Minimal NH_4^+ within the stream and initial conditions of the experiment suggests ammonium generated via mineralization of the sediment is utilized by nitrifying organisms.

5.1 The stream water in this study reflects agricultural- and urban-impacted stream systems that are nitrogen–limited

The stream water in this study is suggested to be reflective of agricultural- and urban-impacted systems that are nitrogen–limited. Measurements and mass balance modelling results suggest dissolved organic matter (DOC=16.40 ± 4.58 mg l^{-1}; DON=0.32 ± 0.23 mg l^{-1}) reflects a mixture of labile terrestrial material, labile autochthonous matter, and a more resistant pool. Nitrate water concentration is moderately high (NO_3-N=2.23 ± 0.01 mg l^{-1}); ammonium water concentration is low (NH_4-N=0.02 ± 0.02 mg l^{-1}); phosphorus water concentration is high (PO_4-P=0.244 mg l^{-1}), reported from Clare, 2019); dissolved inorganic carbon water concentrations are high (DIC-C= 44 ± 0.82 mg l^{-1}); and dissolved oxygen concentration is moderate (DO-O= 6.53 ± 1.03 mg l^{-1}).

We suggest that together the water chemistry agrees with water draining urban and agricultural lands with high background phosphorus levels and in-stream biological

activity. The stream water investigated more broadly can be characteristic of agricultural catchments with moderate intensity practices (e.g., pasture, low-density row crops), urban systems, and mixed land use systems in which phosphorus is non-limiting. For example, we first compare our results with the stream water chemistry found for the South Elkhorn by Clare (2019) and second we compare the results with other literature by using the information found in Clare (2019).

A study examining the Albemarle-Pamlico drainage basin in the southeast Unites States found significant impact to nutrient concentrations in the agricultural and urban mixed-use watershed (Coulter et al., 2004). "They found the highest median total nitrogen concentrations in agricultural and developed basins (2.4 and 1.4 mg N l^{-1}respectively). Mean total phosphorus concentrations was found to be highest in the developed basins (0.36 mg P l^{-1}), and lowest in the agricultural basins (0.16 mg P l^{-1})." (McMahon and Harned, 1998; Coulter et al., 2004). The range of nutrient concentrations found within this similarly mixed-use drainage basin are in agreement with the South Elkhorn Creek system in this study. Previous work has reported that mixed-use watersheds with agricultural land use areas were generally found to have an increased contribution of nutrient concentrations (Dubrovsky et al., 2010; Evans et al., 2014; Koenig et al., 2017; Clare, 2019).

5.2 Fluvial sediment in this study reflects a mixture of terrestrial-derived soil organic matter and aquatic-derived, algal organic matter

The sediment organic matter collected and analyzed in this study is suggested to reflect fluvial sediment transported in rivers and is a mixture of terrestrial-derived soil organic matter and aquatic-derived, algal organic matter. The soil organic matter

component of the sediment likely originates from subsurface soils via gully erosion and to a lesser degree surface soils (Mahoney et al., 2018). Given the sediment's origin, this soil pool likely contains humified soil organic matter and resistant plant organic matter that has undergone carbon oxidation and nitrogen mineralization (Acton et al, 2013). The algal organic matter is believed to reflect a more resistant pool of autotrophs previously undergone degradation of benthic algae matts to coarse and then fine sized organic matter (Ford et al., 2014).

Evidence to support this characterization of fluvial sediment with a mixture of terrestrial and aquatic organic matter stems from previous research in the basin, the experimental design to collect the sediment, the C and N isotope and elemental data results of the sediment, and the modelling incubation results.

Sediment characterization using $\delta^{13}C$, $\delta^{15}N$, SOC, and SN of sediment samples from the uplands and instream suggest a dominance of humified SOM within the upland sediment perhaps as subsurface soil eroded to the stream corridor during an extreme rainfall events. Past research has considered low-order streams draining agricultural lands as dominated by soil organic carbon (Ford and Fox, 2014). The instream sediment chemical composition suggests an increased portion of labile organic matter as algal material and/or fresh plant litter assimilated to the sediment as the amount of SOC is nearly double that of the upland sediment, and has a depleted $\delta^{13}C$ signature relative to terrestrial soils, indicating algal accrual. "Sources of autochthonous and allocthtonous material have been shown to be statistically differentiable with $\delta^{13}C$ ranges of -28 to -42‰ and -10 to -29‰, respectively (Onstad et al., 2000; Palmer et al., 2001; Dalzell et al., 2007; Sakamaki and Richardson, 2011; Schindler Wildhaber et al., 2012; Ford and

Fox, 2015).” Characterization of the sediment reveals a labile (algal) component of the sample collected during an extended low flow period is prevalent as compared to the uplands which is consistent with literature on fluvial sediment organic matter (Dalzell et al., 2007).

The fluvial sediment investigated more broadly bounds fluvial sediment in other studies because: the ‘upland sediment’ is consistent with terrestrial sediment only such as sediment transported in extreme events in mixed-use catchments or sediment transported in steep catchments with no fluvial storage. The ‘in-stream sediment’ is consistent with a mixture of terrestrial and aquatic sediment such as transported during low and moderate hydrologic events in low and moderate gradient mixed-use catchments with fluvial storage.

5.3 Aerobic incubation data and modelling results suggest a moderately active system controlled by dissolved- and sediment-carbon oxidation, CO_2 evasion, nitrogen mineralization, and nitrification

Seventy day aerobic incubation data and modelling results of the sediment substrate in stream water at 25°C suggest a moderately active system dominated by dissolved- and sediment-organic carbon oxidation, CO_2 evasion, nitrogen mineralization, and nitrification. More broadly results suggests even the most resistant fluvial sediment substrate is not inert, despite contention in some circles that this class of mainly terrestrial organic matter with an aggregate diameter of less than 53 μm is passive in freshwater cycles. Reactivity of dissolved constituents in general supports the current paradigm for DOM turnover, carbon supersaturation and nitrification in waters of agricultural- and urban-impacted streams.

The laboratory incubations of the fine sediments suggest the open systems allow for faster decomposition of the carbon and nitrogen within the sediment as compared to the closed systems; this is physically plausible because the open system had approximately three times as much dissolved oxygen as the closed system. In the open flasks, carbon shows a loss of 8-12% whereas the closed flasks shows a loss of 3-5%. The sediment nitrogen in open flasks decreased by 17-25% and for closed flasks by 7-15%.

Comparing the organic matter loss via decomposition/mineralization for the two types (upland and instream) sediments shows conflicting results. The instream sediment, characterized to have more labile organic matter, shows a greater amount of N mineralization. Results indicate 15-25% is lost as compared to 7-17% of N mineralized in the upland sediment, in agreement with literature on refractory and labile organic matter degradation (Hulthe et al., 1998). The comparison of organic C decomposition for the two types of sediments shows results contrary to that of nitrogen mineralization. The instream sediment incubations suggest a carbon loss of 3-8%, whereas the upland incubations show an increased carbon loss of 5-13%. Data results show little (but not inert) decomposition and mineralization of the sediment throughout the incubation period, on the order of 7-25% loss of nitrogen and 3-13% loss of carbon.

Kinetic rates for the oxidation of organic carbon and nitrification were computed using a first-order mass balance model. Kinetic rates for the oxidation of organic carbon are split into two pools based on their reactivity to degradation. The model is manually calibrated such that modeled results are within best agreement with observed data in the laboratory. Parameter description and model terms are reported in Table 3-7. The modeled first-order rate constants are also reported in Table 3-7.

The rate of sediment organic carbon oxidation is modelled from a range of k = 0.0005 – 0.006 day^{-1} (see Figure 5-1 and 5-2). The more reactive algal pool of the sediment organic carbon is on the order of one magnitude greater than the humified soil organic matter pool. This agrees well with previous findings of leaf litter and algae as labile carbon sources decomposing on the order of 10^{-3} to 10^{-2} day^{-1} (Sinsabaugh et al., 1994; Webster et al., 1999; Alvarez and Guerrero, 2000; Jackson and Vallaire, 2007; Yoshimura et al., 2008). The modelled rate constants for the soil organic matter pool are overestimated in comparison with previously reported literature on soil decomposing on the order of 10^{-5} day^{-1} (Webster et al., 1999; Six and Jastrow, 2002), which agrees with our original classification of the sediment being dominated by a soil organic matter pool. Our results suggest this class of sediment degrades similarly to different types of soil, albeit at a smaller rate at the high end of reported values. The rate of carbon oxidation within the open systems is notably about twice that of the closed system, which is attributed to the availability of dissolved oxygen in the open (DO= 6.53 ± 1.03 mg l^{-1}) and closed flasks (DO= 2.61 ± 1.65 mg l^{-1}). The first order rate constants presented with sediment nitrogen is identical to that of carbon oxidation. This is because the mass balance model calculates the rate of sediment carbon oxidation, and then couples those rates to sediment nitrogen using C/N ratios of the measured sediment and estimated sediment pools. These k-values seem to underestimate nitrogen mineralization kinetics in comparison to previous studies on soils, which range from of 0.005 – 0.014 day^{-1} (Stanford and Smith, 1972; Campbell et al., 1981; El Gharous et al., 1990).

Dissolved organic carbon oxidation occurs in all experimental systems and follows a two-pool model formulation similar to sediment. The more labile and reactive

pool has rate constants from 0.03-0.05 day^{-1} (see Figure 5-3). The reactive material makes up the majority of the fresh DOM composition and is preferentially decomposed through the first few weeks of incubation. The modelled k-values for the reactive DOM are comparable to the decomposition rates reported for other labile sources of organic carbon (Sinsabaugh et al., 1994; Webster et al., 1999; Alvarez and Guerrero, 2000; Jackson and Vallaire, 2007; Yoshimura et al., 2008). Once the reactive pool has gone through a stage of decomposition, it behaves akin to the more resistant pool of DOM. This idea is also supported by a similar trend in the modelled C/N ratio of the DOM. The rate constants modelled for the resistant pool of DOM is 0.002 day^{-1}, which is most similar to the rate of sediment decomposition in this study, as well as soil decomposition in others (Webster et al., 1999; Six and Jastrow, 2002). The modelled rate constants for the reactive pool is over one magnitude greater than that of the resistant pool.

The first order rate constant modelled for nitrification is consistent across all experiments with k = 0.2 day^{-1} (see Figure 5-4).The rate of nitrification is impacted by the amount of ammonium in water as well as temperature of the solution. All flasks being consistent in stream water content and environmental conditions allows for a plausible justification of equal rates of nitrification in all experiments. Additionally the availability of ammonium is relatively similar in all systems, with minor differences coming from ammonification of organic matter. The nitrification rates modelled in this study are in good agreement with other studies on the kinetics of nitrification (Ryzhakov et al., 2008; Husic et al., 2020). The rates determined by the study performed by Ryzhakov et al. are a range of 0.22 – 0.68 day^{-1}. Similarly, the study developed by Husic et al. model a calibrated range of 0.12 – 0.5 day^{-1} for first-order nitrification kinetics. Notably our

modelled k-values are on the low end for the nitrification process, potentially explained by external factors effecting the field models presented by other authors, whereas our model is of controlled, incubated flasks.

5.4 Aerobic incubation data and modelling results suggest lack of isotopic enrichment during carbon oxidation, nitrogen mineralization and nitrification

Seventy-day aerobic incubation data and modelling results suggest lack of isotopic enrichment during carbon oxidation, nitrogen mineralization and nitrification. Best estimates of isotope enrichment factors ranged from -3 to +1‰ for dissolved- and sediment-organic matter oxidation, -1 to +1‰ for nitrogen mineralization, and 0.05 to 0.2‰ for nitrification (see Figure 5-5, 5-6 and 5-7). These isotope enrichment results are sparse to nonexistent in the literature for fluvial sediment and suggest fluvial sediment as conservative in terms of its isotope signature during aerobic degradation. The isotope enrichment results generally show agreement with results reported for degradation of sediment cores, and results suggest this theory can be extended to fluvial sediment at least in terms of isotope changes (Mobius, 2013).

Rayleigh modelling results suggest a small enrichment of $\delta^{13}C$ and $\delta^{15}N$ of the sediment via decomposition and mineralization of the organic matter. The enrichment value of these degradation processes are about $-3 \leq \varepsilon \leq +1$‰, in agreement with other studies on the decomposition of organic matter enrichment ranging from ±2‰ (Delwiche and Steyn, 1970; Miyake, 1971; Freyer and Aly, 1975; Mariotti et al., 1981, Casciotti et al., 2003; Kendall et al., 2007; Mobius, 2013). Results of a study on Rayleigh type isotope fractionation of sediment cores during ammonification reveal an enrichment factor between -1.43‰ and-2.3‰ (Mobius, 2013).

If a relatively large amount of ammonium is available, the mineralization process is limited by the nitrification step. The generated nitrate is then strongly depleted in ^{15}N, and will continue to have low $\delta^{15}N$ values if ammonium is present and readily available (Heaton, 1986). However, most of the mineralizable organic nitrogen in the experiments was slowly converted to ammonium. When little ammonium is available the mineralization process is limited by non-fractionating ammonium oxidation, and the nitrate will tend to have an isotopic signature similar to that of organic nitrogen (Heaton, 1986). When the entire amount of ammonium is nitrified to nitrate, both $N^{14}H_4$ and $N^{15}H_4$ molecules are used and neither is preferentially degraded. The situation where ammonification is the rate-limiting step has been shown to dominate in field environment and laboratory incubation soil studies (Delwiche and Steyn, 1970; Mariotti et al., 1981; Mayer et al., 2001). It has been reported in literature $\delta^{15}N_{NH4}$ is within a few permil of the $\delta^{15}N$ of total organic N in sediment due to minimal isotope fractionation (Kendall et al., 2007).

5.5 Analyses of field measurements suggest fluvial sediment is characterized by a single terrestrial soil organic matter pool and isotope values are conservative during high flow events

There is little difference in the sediment data at the two sites for each measurement ($\delta^{13}C$, $\delta^{15}N$, SOC, SN) during high flow ($Q>2.8$ m^3/s) events (see Figure 5-8). This suggests the allochthonous sources can be considered similar throughout the system when comparing drainage areas upstream of Ramsey and upstream of Gage. This tends to agree with previous work in South Elkhorn (Mahoney et al., 2018) because ditches in urban and gullies in agricultural areas are producing sediment. Both of these types of sediment sources dig into the surface and shallow subsurface soil similarly. The

$\delta^{13}C$ and $\delta^{15}N$ of sediment during events of greater magnitude is relatively akin at the midpoint and outlet, which further indicate the allochthonous sources throughout the system are similar for both the upper and lower catchment.

Analyses of field measurements of sediment collected over a three-year period suggest fluvial sediment is characterized by a single terrestrial soil organic matter pool and isotope values are conservative during high flow events. Carbon and nitrogen elemental and isotope values approach constant values as stream water discharge increase to high flow events, and the constant values are equal at multiple sampling locations in the stream network. The sediment organic matter signatures reflect subsurface soils via gully erosion and to a lesser degree surface soils that is composed of humified soil organic matter and resistant plant organic matter that has undergone carbon oxidation and nitrogen mineralization. The constant C and N elemental and isotope values for sediment from different longitudinal stream locations suggest conservative biogeochemical signatures for the sediment during transport during high flow events. The results are consistent with the theory that the uplands of the landscape are highly connected with the stream network during high flow and extreme hydrologic events (Dalzell et al., 2005; Ford et al., 2015; Mahoney, 2017). The results also support the assumption that carbon and nitrogen isotope tracers of sediment can be considered conservative during high flow events.

5.6 Analyses of field measurements suggest fluvial sediment temporarily stored in the streambed accumulates aquatic-derived organic matter that changes the organic signature of the sediment

Analyses of field measurements of sediment collected over the three-year period suggest fluvial sediment temporarily stored in the surficial fine-grained laminae (i.e.,

streambed deposits) accumulates aquatic-derived organic matter that changes the organic signature of the fluvial sediment. Data results of sediment collected during low flow and moderate hydrologic events show increased elemental C and N measurements and slight decreases in isotope C and N measurements over time and across sites (see Figure 5-9). The results are consistent with the concept that temporarily stored fluvial sediment that has terrestrial origin shifts to mixed terrestrial-aquatic organic matter distribution as remnant algal organic matter from benthic algae matts accrues within bed sediment (Fox et al., 2013). The results also support the consideration that carbon and nitrogen isotope tracers of sediment are nonconservative during low and moderate hydrologic events in streams with fluvial deposits, or the in-stream sediment source be treated uniquely.

Sediments collected at both Ramsey and Gage show temporal evidence of algal assimilation during low flows as compared to higher flows because of the increased organic content and isotopic depletion of $\delta^{13}C$ for the sediment (see Figure 5-10 and 5-11). Data results indicate that benthic processes in the streambed are important during extended low flow periods when the streams limited transport capacity allows for deposition and stagnation of particulate matter. The $\delta^{13}C$ of fluvial sediment collected during low peak flow events suggest benthic processes are occurring such as algal assimilation, as the $\delta^{13}C$ ranges from -30 to -40‰ (Ford and Fox, 2015) for algae. Terrestrial litter derived SOM has been shown to be a lower quality source of organic matter relative to organic carbon (Ford et al., 2015). The lower quality carbon of allochthonous SOM has complex compounds such as lignin and cellulose that are more recalcitrant than labile polysaccharides found in autochthonous algal products (Lane et al., 2013; Ford et al., 2015). Low flow events are thought to be indicative of in-stream

sediment organic matter degradation (turnover) and autochthonous integration to the sediment. Benthic processes such as nutrient uptake, sediment mineralization, decomposition, heterotrophic respiration, and algal production are believed to be dominating the sediment organic matter transformations.

The sediment collected provides insight to the sediment response to hydrologic events. The carbon and nitrogen data suggested a dominance of streambed sediment during smaller hydrologic events and increased contribution of upland sediment as the magnitude of peak discharge increases. Extended periods of low flow (>2.8 m^3/s peak discharge) allow sediment to deposit on the streambed and remain until a hydrologic event of great enough magnitude dislodges sediment from the bed. During these stagnant periods, the ability for algal production increases due to settling autotrophic organisms coupled with the desire to utilize carbon dioxide and respire labile organic carbon.

The stable carbon isotope data supports the hypothesis of increased instream algal production during periods of low flow as compared to larger hydrologic events. The mean $\delta^{13}C$ signature observed during low flow events is nearly 0.3‰ depleted relative to the midpoint (Ramsey) of the watershed to the outlet. Assuming external inputs are negligible or limited during these low flow periods allows us to focus on internal, or instream processes that impact the composition of sediment organic matter within the stream. The integration of autochthonous algal carbon is believed to be the reason for a negative shift in $\delta^{13}C$, but the effects may be masked due to the coupled decomposition and mineralization processes within the sediment. The oxidation of organic carbon and ammonification of sediment nitrogen by heterotrophic organisms occur through the water and bed of the stream system. Sediment organic carbon data observed during spring low

flow events has a mean SOC degradation from the midpoint to the outlet. This degradation indicates the amount of organic carbon decomposed is greater than the amount of organic carbon assimilated via algal production. The opposing carbon isotope trend occurs because the fractionation associated with autochthonous integration is prevalent, while the decomposition of organic carbon is known to show little fractionation effects (Delwiche and Steyn, 1970; Miyake, 1971; Freyer and Aly, 1975; Mariotti et al., 1981, Casciotti et al., 2003; Kendall et al., 2007).

5.7 Field measurements of fluvial sediment collected in summer months show agreement with the aerobic incubation study dominated by carbon oxidation, nitrogen mineralization, and nitrification

Analyses of field measurements of sediment collected from multiple longitudinal stream sites during low to moderate hydrologic events in summer months show closest agreement with the seventy day aerobic incubation study dominated by carbon oxidation, nitrogen mineralization, and nitrification. Comparison of sediment data collected from two sites along the streams pathway show a mean 30% decrease in elemental data while isotope values show little to no shift (see Figure 5-12). The field results reinforce the concept that fluvial sediment is moderately active biologically in streams, despite its recalcitrant assertion and typical consideration of tracer conservativeness for the less than 53 μm diameter size class of sediment (Davis and Fox, 2009). The field results also reinforce C and N isotope signatures of fluvial sediment are rather conservative during degradation processes in temporarily storage stream deposits.

Sediment collected at both Ramsey and Gage indicate spatial evidence of carbon oxidation as SOC is decreasing downstream. The data during low flow periods suggests a different trend from Ramsey to Gage noted for high flows, a loss of carbon is indicated to

occur in the bed of the creek via oxidation of the organic matter. This is most evident in summer low flows, where sediment loses about 30% of its organic carbon from Ramsey to Gage. It is expected the stagnant flow and warm temperatures allow for increased deposition of sediment as well as increased microbial activity and therefore more efficient decomposition.

Because seasonal patterns are typically related to temperature and light availability, biological processes are expected to be a driving force behind seasonal variations. Sediment organic carbon (SOC) and sediment nitrogen (SN) indicate that autotrophy contributes most in the warm summer months, and less in the cold winter months. The ability for autochthonous production in the stream is largely dependent on temperature and flow conditions. Therefore, we expect a larger portion of the sediment to contain organic matter in the summer when autotrophy is dominant, compared to colder periods during winter and early spring.

Results of the incubation study are in best agreement with field data collected during low flow periods of the summer months in 2014-2017. The mean $\delta^{13}C$ values from Ramsey to Gage shows an isotopic depletion of less than 0.5‰, similar to the minimal isotope fractionation observed in the lab study. The results of the field SOC data do indicate oxidation of organic carbon, as there is about a 2% depletion of carbon downstream. This suggests the organic carbon is decomposed as it travels through the stream, but is not being significantly altered isotopically, similar to the laboratory incubations.

Further agreements within the field observations and laboratory incubations are in the constant C/N ratio of the sediment. A consistent C/N ratio from Ramsey to Gage as well

as in the incubations suggest that neither carbon or nitrogen is being preferentially oxidize, as in, both are degraded at similar rates.

Figure 5-1: Laboratory incubation data and modeling results of sediment organic carbon and its first-order oxidation rates for all experimental systems

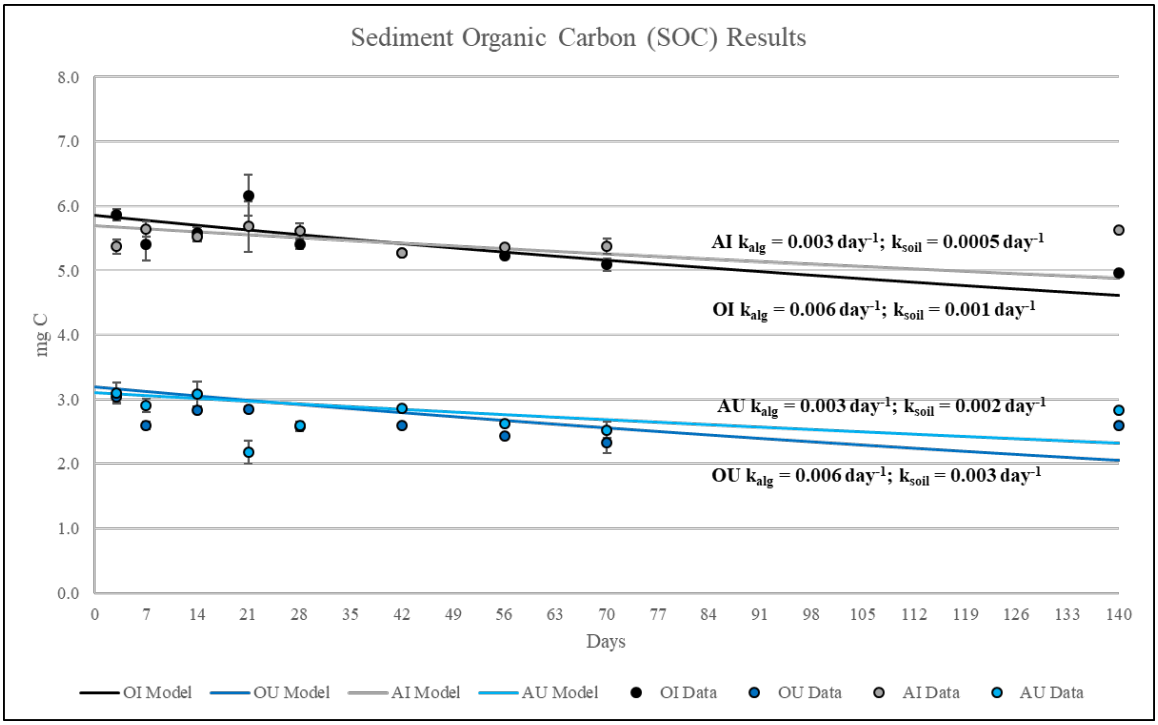


Figure 5-2: Laboratory incubation data and modeling results of sediment nitrogen and its first-order oxidation rates for all experimental systems

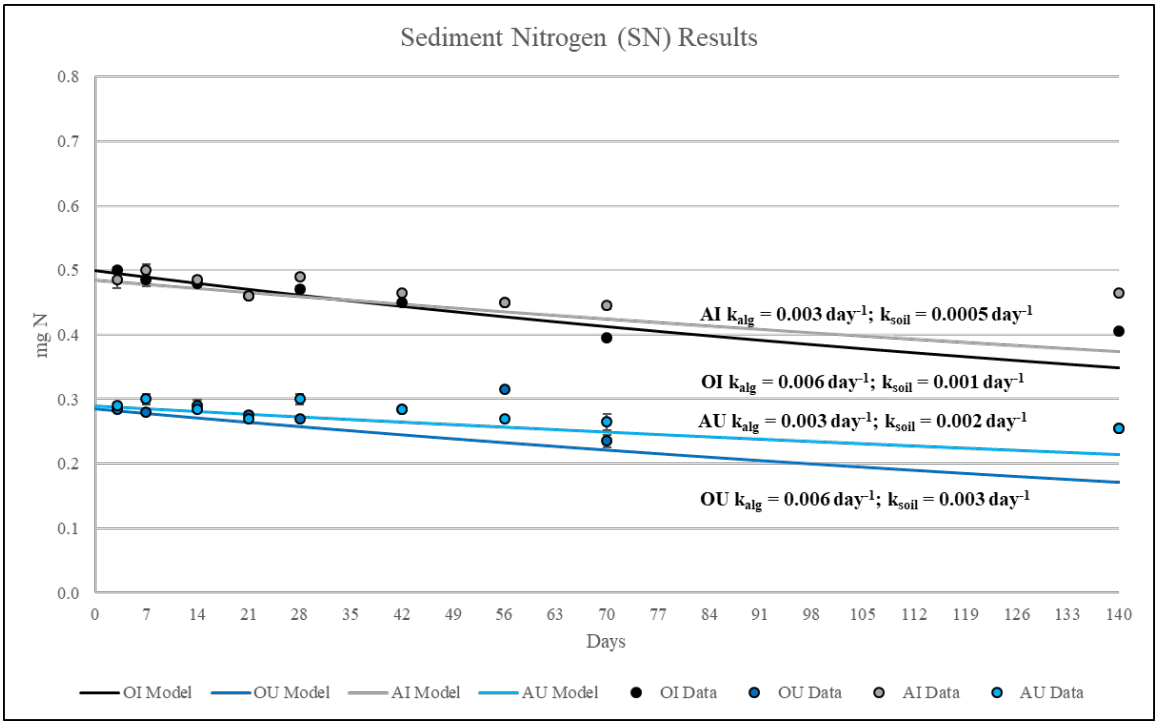


Figure 5-3: Laboratory incubation data and modeling results of dissolved organic carbon and its first-order oxidation rates for all experimental systems

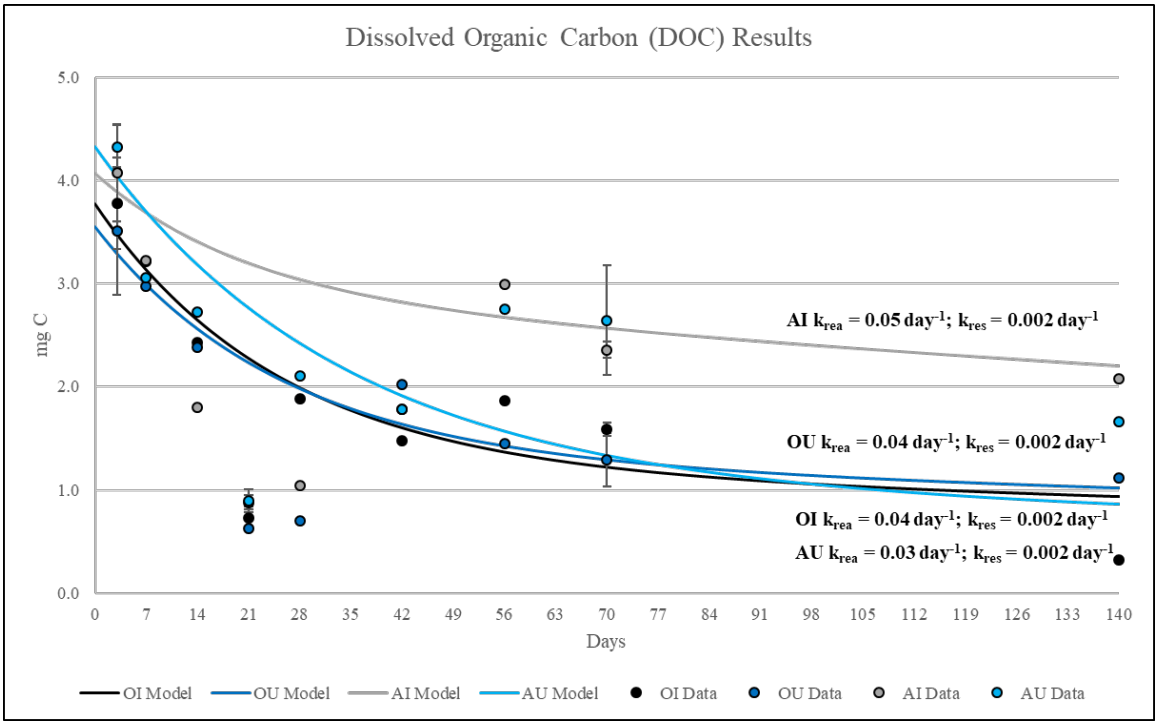


Figure 5-4: Laboratory incubation data and modeling results of nitrate and its first-order nitrification rates for all experimental systems

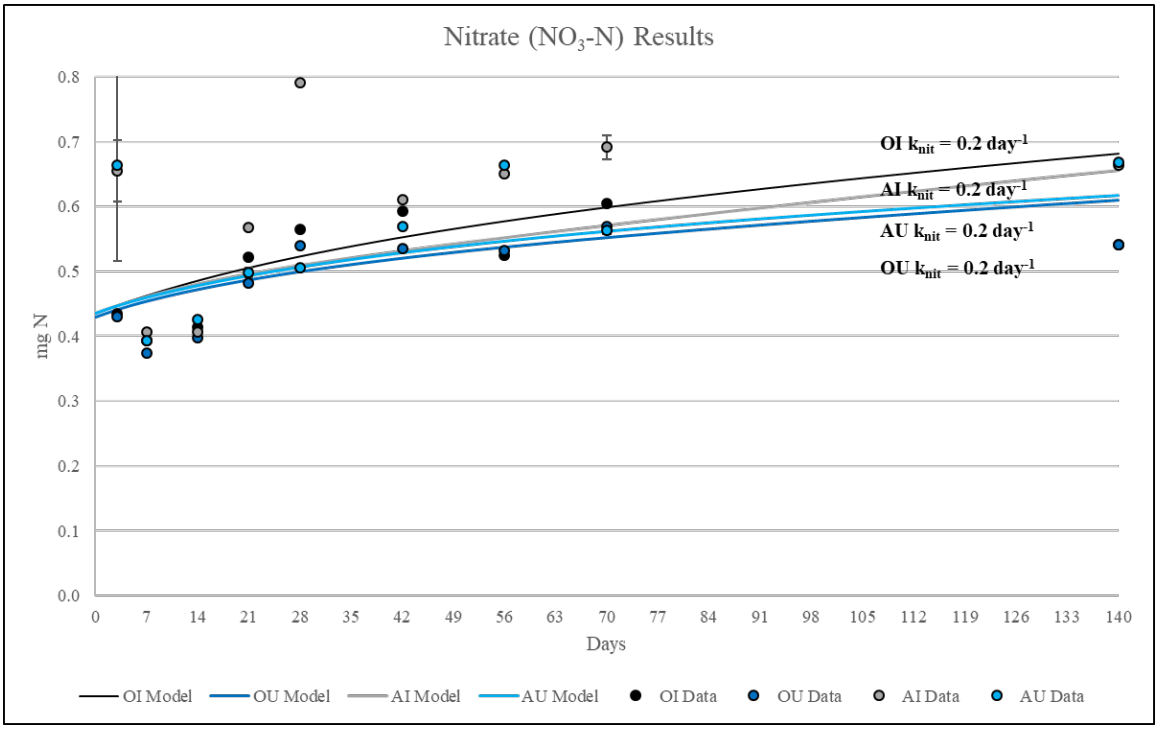


Figure 5-5: Laboratory incubation data and modeling results $\delta^{13}C$ of sediment and its oxidation enrichment rate for all experimental systems

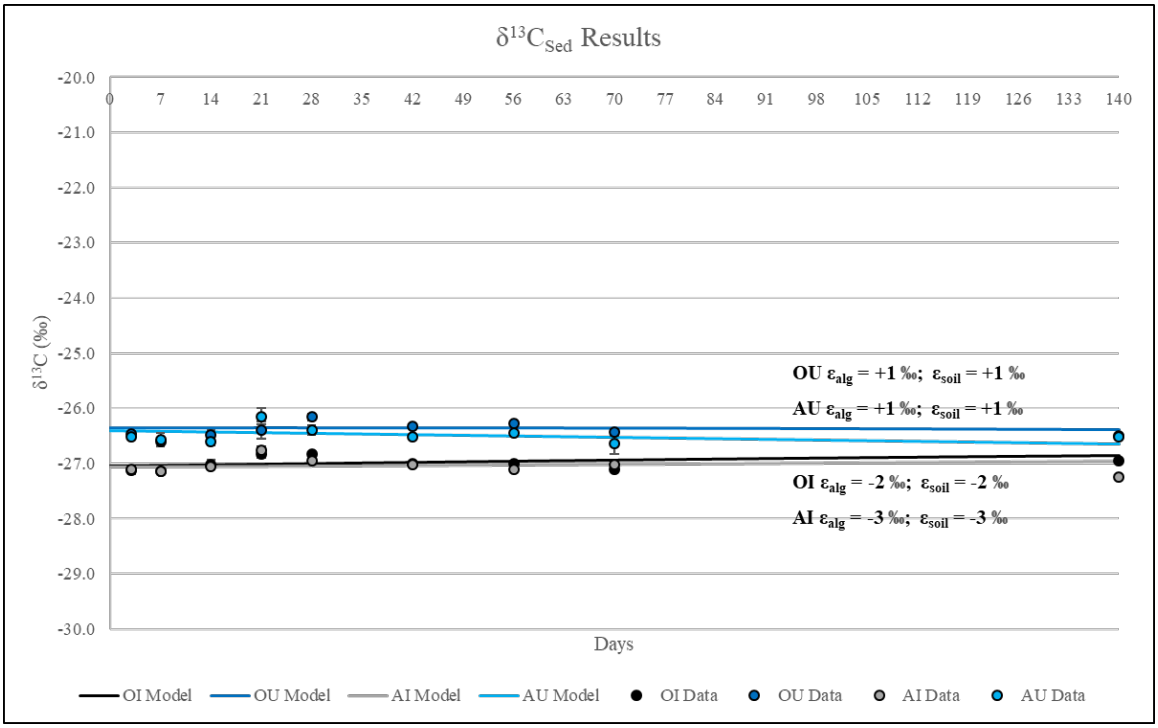


Figure 5-6: Laboratory incubation data and modeling results $\delta^{15}N$ of sediment and its mineralization enrichment rate for all experimental systems

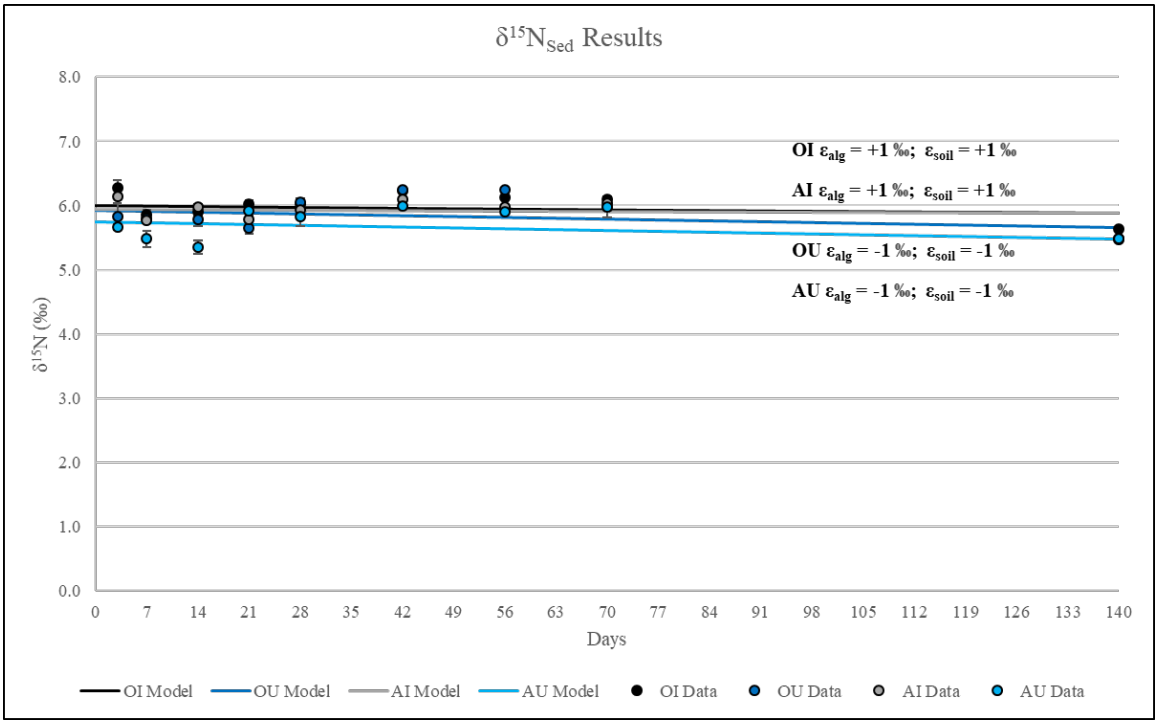


Figure 5-7: Laboratory incubation data and modeling results $\delta^{15}N$ of nitrate and its nitrification enrichment rate for all experimental systems

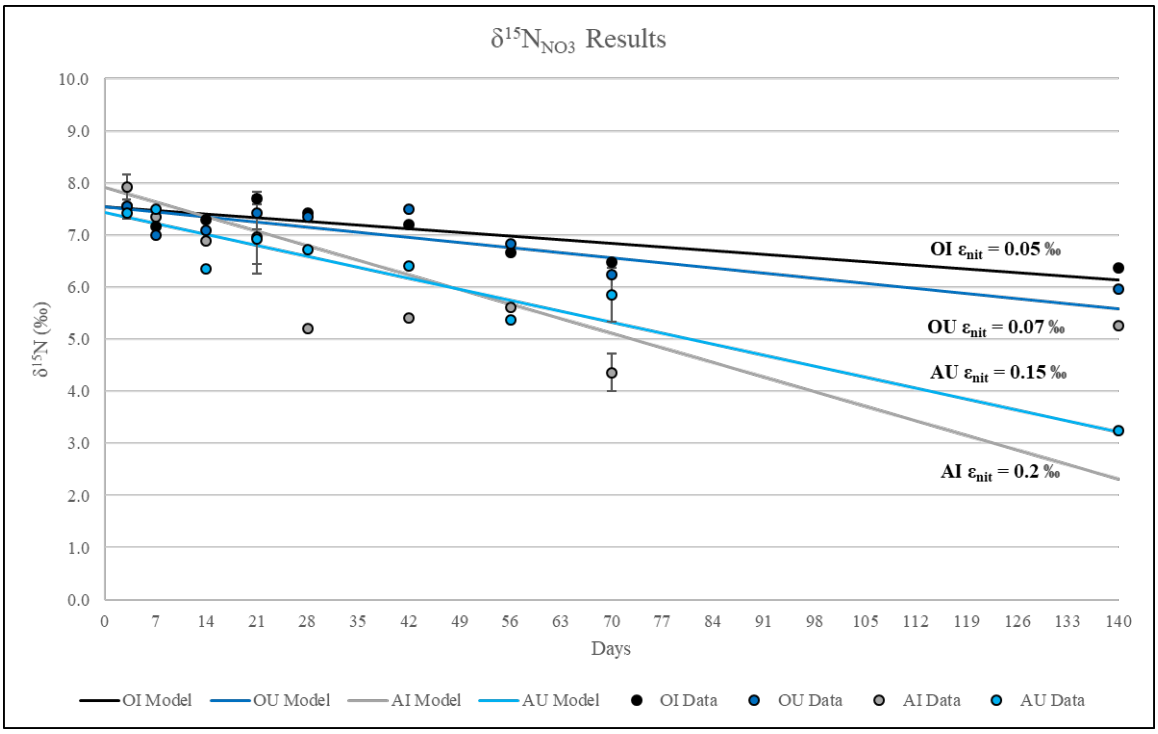
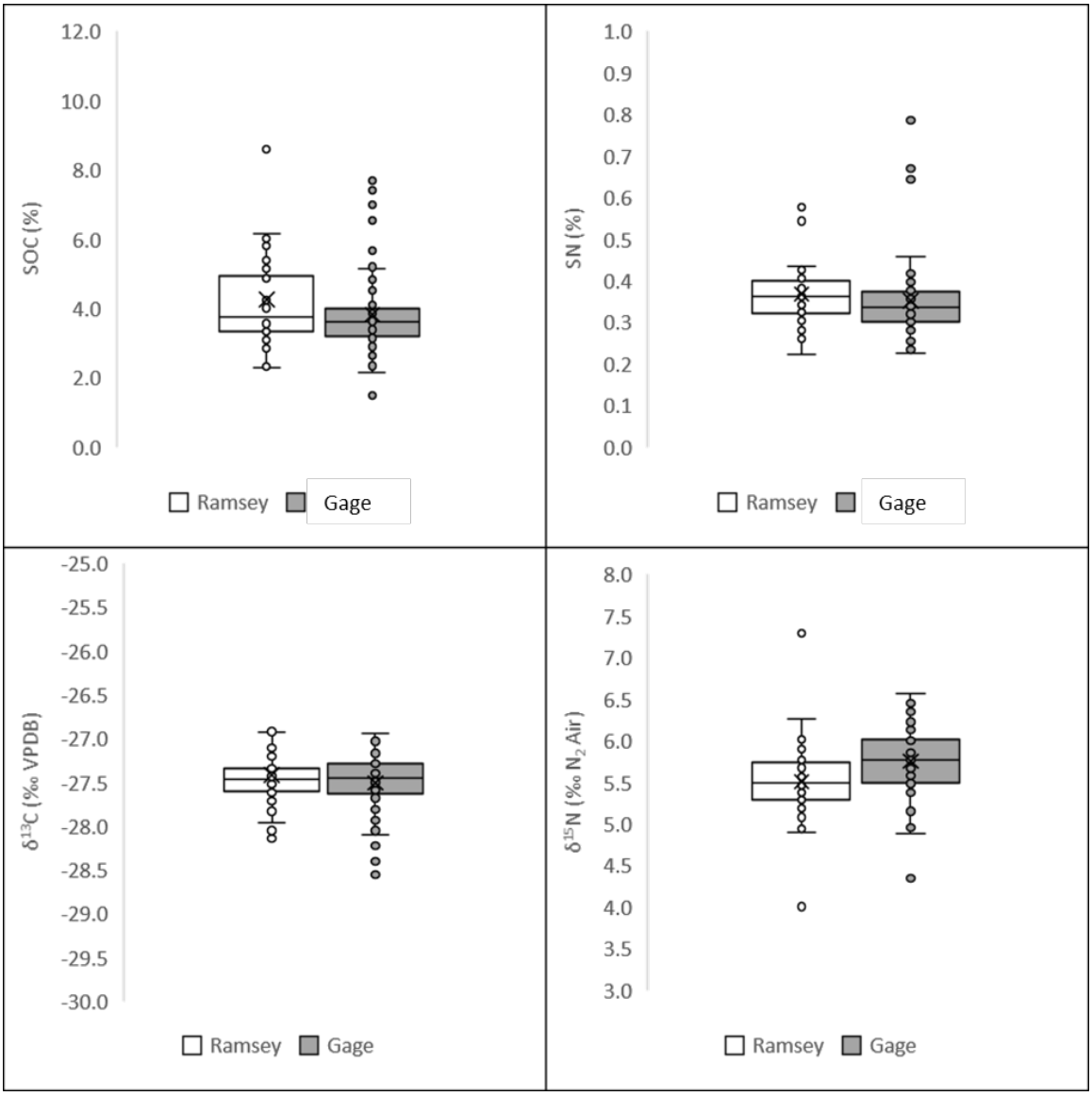
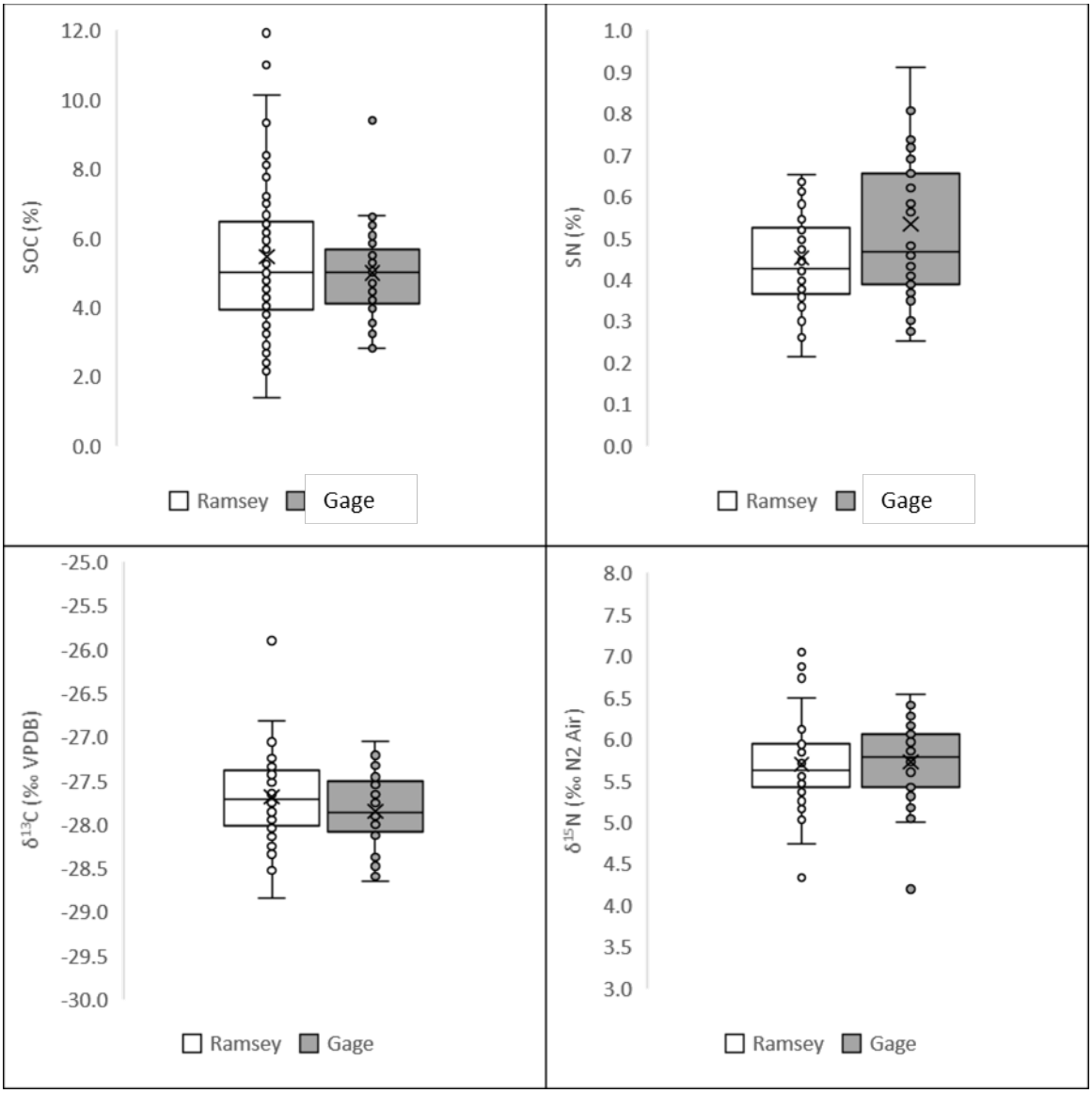


Figure 5-8: Fluvial sediment collected during high peak flow events



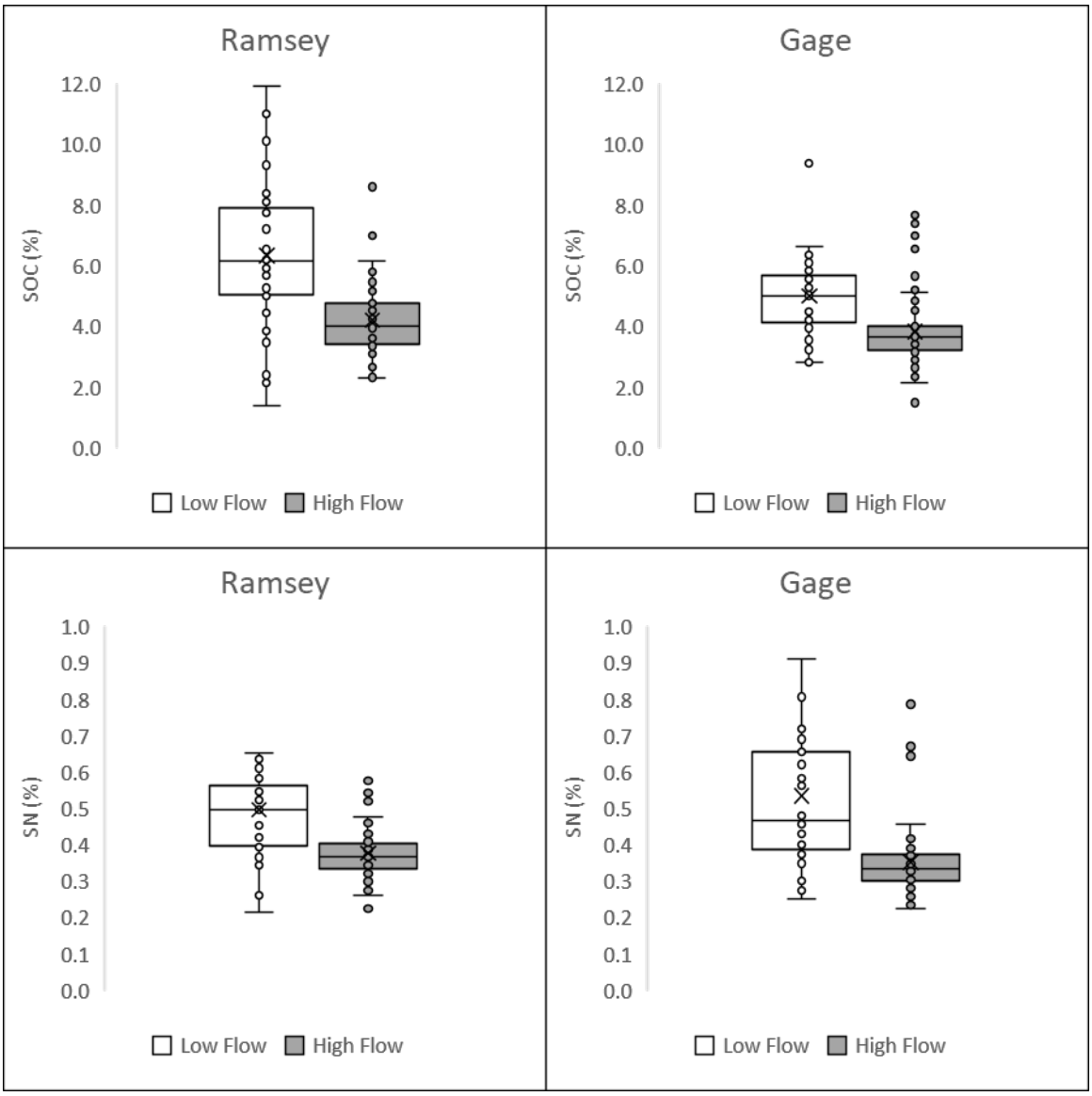
Fluvial sediment data results collected over a three-year period (2014-2017) from South Elkhorn Creek. Sediments were collected during mid to high peak flow events (Q>2.8 m^3/s). Samples were collected at the midpoint (Ramsey) and outlet (Gage) of the South Elkhorn watershed. The elemental percentage (SOC, SN) and the isotope values ($\delta^{13}C$, $\delta^{15}N$) are reported.

Figure 5-9: Fluvial sediment collected during low peak flow events



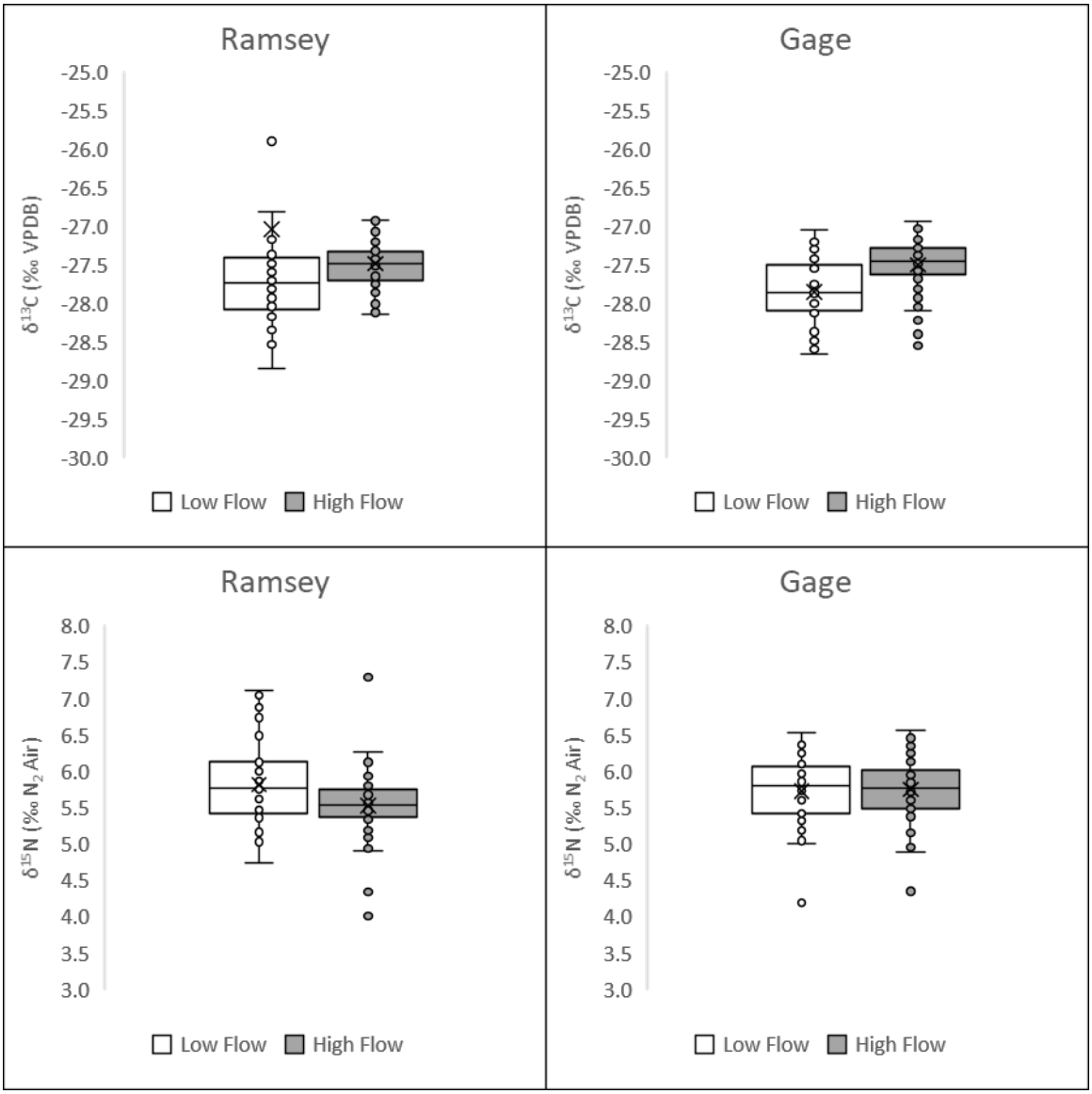
Fluvial sediment data results collected over a three year period (2014-2017) from South Elkhorn Creek. Sediments were collected during low peak flow events ($Q<2.8\ m^3/s$). Samples were collected at the midpoint (Ramsey) and outlet (Gage) of the South Elkhorn watershed. The elemental percentage (SOC, SN) and the isotope values ($\delta^{13}C$, $\delta^{15}N$) are reported.

Figure 5-10: SOC and SN collected at Ramsey and Gage



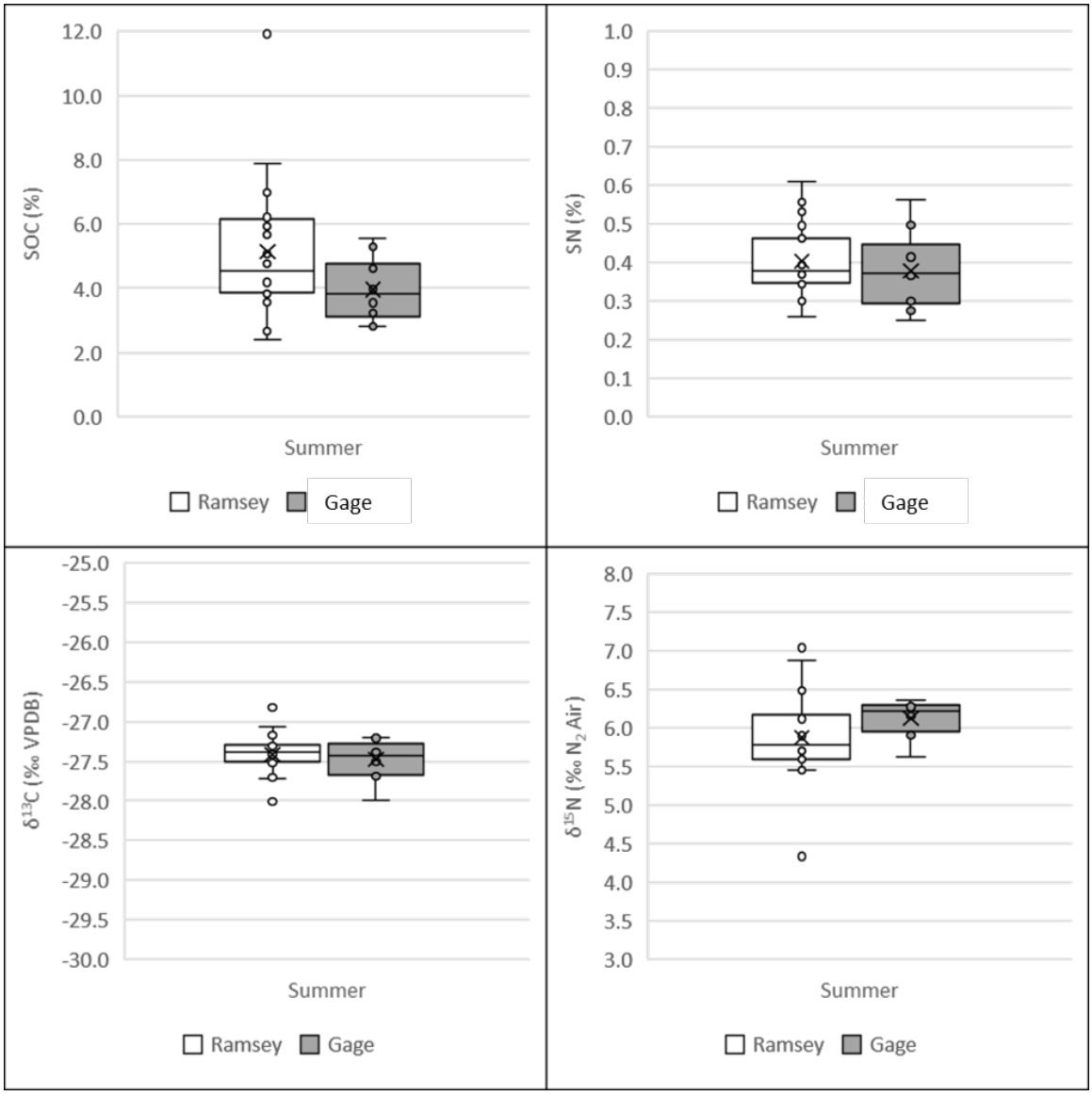
Fluvial sediment data results collected over a three-year period (2014-2017) from South Elkhorn Creek. Samples were collected at the midpoint (Ramsey) and outlet (Gage) of the South Elkhorn watershed. The elemental percentage (SOC, SN) during low peak flow events ($Q<2.8$ m^3/s) and high peak flow events ($Q>2.8$ m^3/s) are reported.

Figure 5-11: $\delta^{13}C$ and $\delta^{15}N$ collected at Ramsey and Gage



Fluvial sediment data results collected over a three-year period (2014-2017) from South Elkhorn Creek. Samples were collected at the midpoint (Ramsey) and outlet (Gage) of the South Elkhorn watershed. The isotope values ($\delta^{13}C$, $\delta^{15}N$) during low peak flow events ($Q<2.8$ m^3/s) and high peak flow events ($Q>2.8$ m^3/s) are reported.

Figure 5-12: Fluvial sediment collected during low peak flow periods in summer months



Fluvial sediment data results collected over a three year period (2014-2017) from South Elkhorn Creek during summer months. Samples collected during low peak flow events ($Q<2.8\ m^3/s$) are reported. Samples were collected at the midpoint (Ramsey) and outlet (Gage) of the South Elkhorn watershed. The elemental percentage (SOC, SN) and the isotope values ($\delta^{13}C$, $\delta^{15}N$) are reported.

Chapter 6 - Conclusion

The conclusion of this thesis is as follows:

1. The stream water in this study reflects agricultural- and urban-impacted systems that are nitrogen–limited. Measurements and mass balance modelling results suggest dissolved organic matter (DOC=16.40 ± 4.58 mg l^{-1}; DON=0.32 ± 0.23 mg l^{-1}) reflects a mixture of labile terrestrial material, labile autochthonous matter, and a more resistant pool. Nitrate water concentration is moderately high (NO_3-N=2.23 ± 0.01 mg l^{-1}); ammonium water concentration is low (NH_4-N=0.02 ± 0.02 mg l^{-1}); phosphorus water concentration is high (PO_4-P=0.244 mg l^{-1}); dissolved inorganic carbon water concentrations are high (DIC-C= 44 ± 0.82 mg l^{-1}); dissolved oxygen concentration is moderate (DO-O= 6.53 ± 1.03 mg l^{-1}); and together the water chemistry agrees with water draining urban and agricultural lands with high background phosphorus levels and in-stream biological activity. The stream water investigated more broadly can be characteristic of agricultural catchments with moderate intensity practices (e.g., pasture, low-density row crops), urban systems, and mixed land use systems in which phosphorus is non-limiting.

2. The sediment organic matter collected and analyzed in this study reflects fluvial sediment transported in rivers and is a mixture of terrestrial-derived soil organic matter and aquatic-derived, algal organic matter. The soil organic matter component of the sediment likely originates from subsurface soils via gully erosion and to a lesser degree surface soils. This soil pool contains humified soil

organic matter and resistant plant organic matter that has undergone carbon oxidation and nitrogen mineralization. The algal organic matter reflects a more resistant pool of autotrophs previously undergone degradation of benthic algae matts to coarse and then fine sized organic matter. Evidence to support this characterization stems from previous research in the basin, the experimental design to collect the sediment, the C and N isotope and elemental data results of the sediment, and the modelling incubation results. The fluvial sediment investigated more broadly bounds fluvial sediment in other studies because: the 'upland sediment' is consistent with terrestrial sediment only such as sediment transported in extreme events in mixed-use catchments or sediment transported in steep catchments with no fluvial storage; and the 'in-stream sediment' is consistent with a mixture of terrestrial and aquatic sediment such as transported during low and moderate hydrologic events in low and moderate gradient mixed-use catchments with fluvial storage.

3. Seventy day aerobic incubation data and modelling results of the sediment substrate in stream water at 25°C suggest a moderately active system dominated by dissolved- and sediment-organic carbon oxidation, CO_2 evasion, nitrogen mineralization, and nitrification. Oxidation of sediment was higher than previously reported ranges, albeit estimates of fluvial sediment oxidation are somewhat sparse in the literature. DOC oxidation is consistent with reported values of the decomposition of labile sources of carbon in stream systems; nitrogen mineralization follows C oxidation and disagrees with reported values

for soils, as mineralization of nitrogen is about one magnitude greater than experimental observations; and nitrification rates are constant in all experiments and consistent with other reported ranges for first-order nitrification kinetics. More broadly results suggests even the most resistant fluvial sediment substrate is not inert, despite contention in some circles that this class of mainly terrestrial organic matter with an aggregate diameter of less than 53 μm is passive in freshwater cycles. Reactivity of dissolved constituents in general supports the current paradigm for DOM turnover, carbon supersaturation and nitrification in waters of agricultural- and urban-impacted streams.

4. Seventy-day aerobic incubation data and modelling results suggest lack of isotopic enrichment during carbon oxidation, nitrogen mineralization and nitrification. Best estimates of isotope enrichment factors ranged from -3 to +1‰ for dissolved- and sediment-organic matter oxidation, -1 to +1‰ for nitrogen mineralization, 0.05 to 0.2‰ for nitrification. These isotope enrichment results are fairly sparse to nonexistent in the literature for fluvial sediment and suggest fluvial sediment as conservative in terms of its isotope signature during aerobic degradation. The isotope enrichment results generally show agreement with results reported for soil degradation, and results suggest this theory might be extended to fluvial sediment at least in terms of isotope changes. Isotope enrichment of dissolved inorganic carbon is consistent with theory for evasion of water from rivers supersaturated in CO_2.

5. Analyses of field measurements of sediment collected over a three-year period suggest fluvial sediment is characterized by a single terrestrial soil organic matter pool and isotope values are conservative during high flow events. Carbon and nitrogen elemental and isotope values approach constant values as stream water discharge increase to high flow events, and the constant values are equal at multiple sampling locations in the stream network. The sediment organic matter signatures reflect subsurface soils via gully erosion and to a lesser degree surface soils that is composed of humified soil organic matter and resistant plant organic matter that has undergone carbon oxidation and nitrogen mineralization. The constant C and N elemental and isotope values for sediment from different longitudinal stream locations suggest conservative biogeochemical signatures for the sediment during transport during high flow events. The results are consistent with the theory that the uplands of the landscape are highly connected with the stream network during high flow and extreme hydrologic events. The results also support the assumption that carbon and nitrogen isotope tracers of sediment can be treated as conservative during high flow events.

6. Analyses of field measurements of sediment collected over the three-year period suggest fluvial sediment temporarily stored in the surficial fine-grained laminae (i.e., streambed deposits) accumulates aquatic-derived organic matter that changes the organic signature of the fluvial sediment. Data results of sediment collected during low flows and moderate hydrologic events show increases in elemental C and N measurements and decreases in isotope C and N measurements over time

and across sites. The results are consistent with the concept that temporarily stored fluvial sediment that has terrestrial origin shifts to mixed terrestrial-aquatic organic matter distribution as remnant algal organic matter from benthic algae matts accrues within bed sediment. The results also support the consideration that carbon and nitrogen isotope tracers of sediment be treated as nonconservative during low and moderate hydrologic events in streams with fluvial deposits, or the in-stream sediment source be treated uniquely.

7. Analyses of field measurements of sediment collected from multiple longitudinal stream sites during low to moderate hydrologic events in summer months show closest agreement with the seventy day aerobic incubation study dominated by carbon oxidation, nitrogen mineralization, and nitrification. Comparison of sediment data collected from two sites along the streams pathway show a 30% decrease in elemental data while isotope values show little to no shift. The field results reinforce the concept that fluvial sediment is moderately active biologically in streams, despite its recalcitrant assertion by some authors for the less than 53 μm diameter size class of sediment. The field results also reinforce C and N isotope signatures of fluvial sediment are rather conservative during degradation processes in temporarily storage stream deposits.

Future Work

The research results and discussion in this paper will also be reported in scientific publication. Two journal papers are planned as follows:

I. Paper 1 will focus on the reactivity and isotope enrichment of sediment and dissolved constituents in the incubation study, as well as compare the results qualitatively with the field measurements. We find very few studies of reactivity and isotope enrichment for this class of substrate in stream water despite the fact that these are highly uncertain organic matter pools in C and N freshwater cycles/budgets. Paper 1 will include some results from conclusions 1 through 7.

II. Paper 2 will focus on the conservativeness, or non-conservativeness, of carbon and nitrogen stable isotope tracers used to perform sediment fingerprinting during low, moderate, and high flow hydrologic events. Several overlapping processes potentially impacting conservativeness will be focused on in the paper, including: source (non)conservativeness over time; (non)conservativeness during degradation while sediment is temporarily stored in-stream; (non)conservativeness as aquatic organic matter accrues to sediment stored in-stream; (non)conservativeness during physical sorting and disaggregation of sediment organic matter during transport; and discussion of other physical and biogeochemical processes potentially impacting isotope tracer conservativeness. Paper 2 will be coupled with sediment fingerprinting modelling to understand how shifts and variance associated with non-conservativeness could potentially impact estimates of sediment provenance.

A future project was proposed for the National Science Foundation's graduate research funding program. This proposal is included to highlight a potential direction for this research.

Study of the microbiome's structure and isotope functions when transforming nitrogen in the fluvial system: A Mississippi River Basin study

The microbiome is the microorganisms in a particular environment and I plan to study the fluvial system. The fluvial system is the set of streams and rivers transporting water, sediment and nutrients from the landscape to river deltas[1]. Microbiome's structure and isotope functions for riverbed sediments of the fluvial system will be studied in controlled laboratory experiments. My emphasis will be on denitrifying bacteria identified with 16S rRNA sequencing from river sediment-water samples and cultured in the environmental laboratory to measure isotope fractionation under varying ideal conditions. I will complement my lab experiments with a field study to investigate additional controls in the river including specific surface area of sediment and river turbulence. My goal is to understand how connectivity between the terrestrial and aquatic environments influences and structures microbiomes in the Mississippi River Basin.

I will collect river sediment longitudinally down the Mississippi River system from six locations including a (1) small agricultural stream in Lexington, KY, (2) the Kentucky River, (3) the Ohio River near Paducah, KY, (4) the middle Mississippi south of Memphis, TN, (5) the lower Mississippi near Vicksburg, MS, and (6) the Mississippi River delta south of New Orleans, LA. At each location, I will use a jon boat and USGS standard clam shell grab sampler to collect riverbed sediments from a minimum of 25-(sub)locations.

Hypotheses: My hypothesis (H0) is that the specific bacterial community structure and isotope function of riverbed sediments transitions from a likeness to that of soils in low order streams to a likeness of lake plankton in large slow moving rivers. The reason is the river continuum transitions from terrestrial (soil) organic matter dominance at its headwaters to aquatic organic matter dominance at its deltas[2] and the microbiome follows suit. My alternative hypothesis (HA) is that the specific bacteria form and function of riverbed sediments shows uniqueness to either soil or plankton. The reason is the hybridization of soil and aquatic organic matter early in the river continuum[3,4] make the structure and isotope function of the microbiome unique throughout. The 16S rRNA gene sequences resulting from the samples collected longitudinally throughout the Mississippi River Basin will provide evidence for H0 and HA. The 16S rRNA gene sequences in our samples will be compared against existing databases for bacteria[5]. To provide evidence for H0 or HA in terms of function, the isotope fractionation rates during denitrification by the microbes in samples will be compared against the published rates for soils and lakes[6,7].

Experiments: In order to re-produce quality control of specific bacteria identification and isotope fractionation during denitrification in the laboratory, I will work with pure cultures prior to running analyses on my field samples. Pure cultures will be obtained from the American Type Culture Collections, grown overnight, then harvested and transferred to Erlenmeyer flasks containing a defined growth medium under a range of environmental factors, including temperature and pH. The pure cultures studies are well controlled and will enable us to understand the extent of N fractionation impacted by a variety of microbial species, and provide a reference for the field samples. Next, the collected field sediments from the Mississippi River system will be investigated. I will

isolate and identify denitrifying species from sediments through the standard enrichment, purification, and 16S rRNA sequencing. The isolates will then be examined for their ability to impact N via fractionation. The changes in the elemental (SOC, SN) and isotope ($\delta^{13}C_{sed}$, $\delta^{15}N_{sed}$) values of sediment and water will be measured in a series of batch experiments with varied aerobic and anaerobic (redox) conditions. Three sets of experiments will be carried out to compare aerobic and anaerobic decomposition rates, with an anaerobic system amended using a sulfate additive (200 ppm) to favor sulfate reduction (SO_4^{2-} as terminal electron acceptor) during anaerobic respiration[8]. The dissolved constituents (DOC, DIC, NO_3^-, NH_4^+, o-PO_4^{3-}, & SO_4^{2-}) in each experiment will be analyzed to examine sediment and water exchange. I will develop a numerical model with mass balance and kinetic sub-routines for the incubations that allow estimates of denitrification and isotope fractionation by the microbes.

Complimentary Field Study: I will set up and carry out a field study to compare with my idealized laboratory study in order to connect the microbiome's potential with the real transformations in a river. I will study a 100-meter reach of an agricultural stream (i.e., the small stream in the lab samples), and perform a mass balance for N occurring in the water column and sediment bottom of the stream. I will place SeaBird Coastal Suna V2 nitrogen sensors at the upstream and downstream ends of the 100-meter reach to measure dissolved nitrate on 15-minute time steps. I will use YSI EXO 3 sensors to measure temperature, conductivity, dissolved oxygen, turbidity and pH at the two locations and collect samples on hourly time steps using Tyledyne ISCO pump samples. The water samples will be split and analyzed for NO_3, NH_4, DIC, DOC, PO_4, and the isotope signatures of NO_3 ($\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$). Sediment in the water samples will be analyzed for

$\delta^{13}C$ and $\delta^{15}N$. I will construct and model the microbial-mediated stream N and isotope transformations in the sediments for the reach. The isotope-based numerical models developed for the river-type by Ford and Fox[9,10] will be used as a starting point for my model. Deviations from predicted laboratory rates are likely attributed to limited surface area of contact (i.e., lower rates) or advection of solutes via turbulence (i.e., higher rates). To better understand field controls, I will use an underwater camera with an endoscope to map the streambed sediment surface at a micrometer scale to estimate the available surface area for contact. I will use a Sontek MicroADV velocimetry instrument to measure turbulence characteristics of the flow near the streambed to compare against Sontek measurements taken in the laboratory incubations.

Intellectual Merit: My isotope tracer methods deliver a new application that can be built on by other researchers who study microorganisms form and function in rivers. Pure culture studies will quantify nitrogen transformations for specific microorganisms and identify the bacteria with 16S rRNA sequencing. Numerical modelling of isotope fractionation under ideal conditions of the laboratory study will provide insight for transformations in the field study. The controlled-laboratory component will ensure adequate internal validity and field observations will be interpreted to maintain realistic systems for the incubations. One unique deliverable of my work will be a conceptual model of the Mississippi River Basin microbiome. I will use this visual tool to share my results at conferences and further engage with the community.

Broader Impacts: My ultimate goal is to view the microbiome in terms of isotopes, teaching how to better understand microbiomes structure and transformations in river systems using contemporary technology. I will teach this concept to engineering students

because of its validity to many sectors of civil engineering (e.g., wastewater, public health). As a student I will use my platform to promote collaborative research by working with a wide-range of scientific disciplines and other universities (e.g., Murray State, Mississippi State). My continued involvement with student engagement will allow me to actively recruit high school and undergraduate students to gain valuable research experience on this project. Teaching students about the microbiome in diverse environments will broaden their scientific perspective, while enhancing their ability to interpret and communicate findings with other researchers.

References: **1** Schumm (1997) *Wiley,* **2** Owens et al. (2005) *River research and applications,* **3** Droppo et al. (2005) *Catena,* **4** Fox et al. (2014) *Hydrological processes,* **5** Zwart et al. (2002) *Aquatic microbial ecology,* **6** Heaton (1986) *Chemical Geology,* **7** Kendall (1998) *Isotope tracers in catchment hydrology,* **8** Lehmann et al. (2002) *Geochimica et Cosmochimica Acta,* **9** Ford & Fox (2015) *Water Resources Research,* **10** Ford et al. (2017) *Water Resources Research*

Appendices

Sediment Laboratory Preparation

Adopted and modified from the Cane Run QAPP (Husic, 2018) and South Elkhorn QAPP (Ford, 2014).

A. Settling/Decanting Field Samples

- Bring sediment samples back to lab after collection in the field.
- Leave samples undisturbed in buckets/appropriately-sized containers for 48 hours in refrigerator (Hydrolab basement Floor Raymond Bldg.) set to 4°C.
- 48 hours is a relative time that usually allows all of the sediment contained in the sample to settle to the bottom of the bucket/container. **If all sediment has not settled to the bottom of the bucket, allow more time for settling**.
- Gently pour water off the top of settled sediment samples. If a large volume of water is present, may use small rubber tubing as siphon. This is up to the technician's preference.
- Pour/siphon water from the bucket until either (a) the sediment nearly flows out of the bucket if pouring or (b) the sample has a manageable amount of water to allow for centrifugation.

B. Centrifuging (Bulk Sample)

- Agitate decanted sample in bucket to encourage homogeneous mixture.
- Pour sample into a clean (4 DI/DO rinses) 750 mL Nalgene pitcher until the pitcher is nearly full.
- Place bucket, bottle (in bucket), and bottle cap for a sample on each side of balance.
- Slowly fill one bottle with sample until nearly full (almost to neck).
- Slowly fill opposing tube with sample until nearly balanced.
- Using plastic pipette, delicately balance both bottles with DI/DO H_2O (see *"DI/DO H_2O"* procedure) until the two sides are the same weight.
- Place cap on tube.
- Align these two balanced bottles across from one another in centrifuge.

- Repeat steps 1-7 with remaining two bottles so opposing tubes are well balanced.
- Settings on centrifuge should be set as follows:
    a. *Rotational Velocity:* 4.25 on knob or 4250 rpm
    b. *Time:* 4-7 minutes
    c. *Temperature:* room temp (20 degrees Celsius)
    d. *Rotor:* SH-3000
- Close top (will click).
- Press start button (Play button located to the right of the temperature).
- If vibration is severe upon spinning, samples are not well balanced. Press the stop button (square), inspect tube balance, add DI/DO H_2O, etc.
- After centrifuge is **completely** stopped, centrifuge door light will come on open top by pressing door button.
- Remove adapters/bottles two at a time, decant, and add additional sample from the Nalgene pitcher to each bottle, balancing opposing bottle as necessary.
- Repeat previous steps until the sample is completely centrifuged into four bottle.
- Consolidate entire sample into 1 labeled centrifuge tube (may need to use two centrifuge tubes if the sample contains a large amount of sediment).
- After consolidation, bottle may have a large amount of supernatant above the sediment. If this occurs, place the single centrifuge bottle back into the cooler until another sample is centrifuged and contains a large amount of supernatant as well. These two separate samples can be balanced, centrifuged, and decanted to remove excess supernatant.
- Place bottles in freezer (-40°C) after removing as much supernatant as possible.

Notes:

- If, after spinning, sample has a large amount of fine sediment still in suspension (murky color), add ~10mL Magnesium Chloride Hexahydrate ($MgCl_2$-$6H_2O$) prepared at 0.5M (see *"Magnesium Chloride"* procedure).
- Once the entire sample is poured into the Nalgene pitcher, spray off any sediment remaining on the inside of the bucket using DI/DO H_2O.

- Once the entire sample is poured into the centrifuge tubes, spray off any sediment remaining on the inside of the Nalgene pitcher using DI/DO H_2O.

C. Freeze Drying

- Check to make sure there is enough oil in the machine. (Look in the front at the tube).
- Turn on the refrigeration unit by pressing the button that says "Fridge". (It is preferred to do this a little before the samples are put in so that the atmosphere will cool faster.)
- This procedure differs depending on the size of the bottle. If the sample bottle fits in the glass jars, refer to section 1. If the sample bottle does not fit in the glass jars refer to section 2.

Section 1:

- Be sure that the sample bottle is covered with cheesecloth and held with a rubber band.
- Start the vacuum, by pressing the button on the front of the Freeze drier that says, "Pump". (don't turn on pump until fridge temperature <-41C)
- Place a sample bottle into the glass jar and seal the jar with the rubber cap.
- Push the cap firmly into the vacuum chamber and ensure that it is on tightly so that the glass jar does not fall off.
- Turn the valve on the manifold from "Vent" to "Vac" to allow a vacuum to reach the sample.
- Make sure the drain hose is removed and that all the pressure releases are closed.

Section 2:

- Be sure that the sample bottle is covered with cheesecloth and held with a rubber band.
- Remove the top glass piece from the vacuum chamber.
- Place the sample bottles inside the chamber around the edge so that they are stable. (put samples with the most ice on top)
- Put the top glass piece into its proper position. Be sure that there is a good seal.

- Make sure the drain hose is removed and that all the pressure releases are closed.
- Start the vacuum, by pressing the button on the front of the Freeze drier that says, “Pump”. (don’t turn on pump until fridge temperature < -41C)

Once the samples are dry:

- Once samples are completely dry, turn off the vacuum by pressing the “Pump” button on the freeze drying unit.
- Slightly turn a pressure release so that pressure is slowly restored to atmospheric pressure.
- Remove glass piece or the jars to remove the samples.
- Recap the samples.
- a) If samples are going to be put on to the freeze dryer right away and the condenser does not have a lot of ice on it, leave the condenser on. Repeat the previous steps for more samples.

b) If not, turn the condenser off by pressing the same button that was used to turn it on. Be sure drain valve is open. Let the condenser drain until all of the ice is off the side wall.

D. <u>Consolidation and Weighing</u>

a. This is a dry procedure so all equipment used must be washed and acetone used to ensure dryness.

b. Weigh an empty Nalgene bottle and record the empty weight.

c. Using the spatula, break large soil particles into smaller particles so that they can be wet sieved easier.

d. Tip the centrifuge bottle into the Nalgene bottle (a funnel may be needed).

e. Using the spatula, scrape the side of the centrifuge tube so all soil particles fall to the bottom.

f. Tip the centrifuge bottle into the Nalgene bottle.

g. Using the spatula strongly tap the centrifuge bottle so that all of the soil gets knocked into the Nalgene bottle.

h. Repeat the three previous steps until all of the sediment is in the Nalgene bottle.

i. Weigh the Nalgene bottle with the sample and record the weight.

j. Label the Nalgene bottle with the appropriate name and number.

E. Wet Sieving

a. Use DIDO water to fill the Nalgene bottle and shake the bottle to break up particles.

b. Pour sediment solution through 3" diameter 53 micron sieve. Flush through sieve with DIDO water into sieve pan. (It helps to shake the sieve as you spray the sieve.)

c. Rinse bottom of 53 micron sieve with DIDO water into sieve pan. Repeat these two steps until water on top and bottom while washing remains clear.

d. Rinse fine solids retained on 53 micron sieve through plastic funnel leading to centrifuge tube (labeled w/sample #).

e. Pour contents of pan through funnel into separate centrifuge tube (labeled w/sample #).

f. **Rinse funnel (4 DI/DO, 1 acetone) between each sample.**

g. Each sample should now be split into two parts (>53µm, <53µm) and labeled accordingly.

h. Keep samples in labeled bucket in ERTL refrigerator (3rd Floor) until centrifugation.

F. Centrifuging (Wet Sieved Sample)

a. Agitate decanted sample in bucket to encourage homogeneous mixture.

b. Pour sample into a clean (4 DI/DO rinses) 250 mL Nalgene pitcher until the pitcher is nearly full.

c. Place bucket, tube (in bucket), and tube cap on each side of balance.

d. Slowly fill one tube with sample until nearly full (almost to neck) **Avoid any liquid on outside of tube or on insert (use pipette if necessary) if any fluid is on side of tube or insert dry before placing in centrifuge**.

e. Slowly fill opposing tube with sample until nearly balanced.

f. Using plastic pipette, delicately balance both tubes with DI/DO H_2O (see *"DI/DO H_2O"* procedure) until the two sides are the same weight.

g. Place cap on tube.

h. Align these two balanced tubes across from one another in centrifuge.

i. Repeat steps 1-7 with remaining two tubes so opposing tubes are well balanced.

j. Settings on centrifuge should be set as follows:

   i. *Rotational Velocity:* 3200 * g

   ii. *Time:* 4 minutes 0.04 = 4 minutes 4.00 = 4 hours

   iii. *Temperature:* room temp (20 degrees Celsius)

   iv. *Motor:* 243 – Rotor

   v. *Acceleration (on left):* 3

   vi. *Brake (on right):* 2

k. Close top gently will self set (will click).

l. Press start button (Play button located to the right of the temperature).

m. If vibration is severe upon spinning, samples are not well balanced. Press the stop button (square), inspect tube balance, add DI/DO H_2O, etc.

n. After centrifuge is **completely** stopped (0*g, centrifuge will beep and say "end"), open top by pressing appropriate button.

o. Remove adapters/tubes two at a time, decant, and add additional sample from the Nalgene pitcher to each tube, balancing opposing tubes as necessary.

p. Repeat previous steps until the sample is completely centrifuged into four tubes.

q. Consolidate entire sample into 1 labeled centrifuge tube (may need to use two centrifuge tubes if the sample contains a large amount of sediment).

r. After consolidation, tubes may have a large amount of supernatant above the sediment. If this occurs, place the single centrifuge tube back into the cooler until another sample is centrifuged and contains a large amount of supernatant as well. These two separate samples can be balanced, centrifuged, and decanted to remove excess supernatant.

s. Place tubes in freezer (-40°C) after removing as much supernatant as possible.

G. <u>Consolidation and Weighing</u>

a. Samples are again consolidated and weighed as in Step D

H. <u>Grinding</u>

a. Place the steel ball into the vial with.

b. Fill the stainless steel vial for the Wig-L-Bug grinder roughly halfway with sample using the funnel with the small opening. Be sure to scrape the funnel to ensure all the soil is in the vial. For soils, this volume is approximately equal to 1 gram of sample. For organics, this weight is much less. Place the cap on.

c. Secure the vial in the arms of the grinder. Make sure that the top of the vial is facing the rear of the grinder (towards the brass nut). Tighten the front screw using the provided allen wrench (two turns past hand tight is sufficient).

d. Run the Wig-L-Bug for 30 seconds.

e. Once the grinder has stopped, loosen the front screw and remove the vial.

f. Place the ground sample into the desired container.

g. Using a magnetic-tipped screwdriver, remove the steel ball from the vial.

h. If more ground sample is required, repeat steps 1-8.

i. Be sure to clean the equipment thoroughly between each sample. Consecutive runs of the same sample do not require cleaning the equipment. Follow the procedure below for each instrument:

    i. Tap water rinse/wire brush scrub

    ii. 4 DI/DO rinses

    iii. 1 100% ethanol rinse or acetone

    iv. Dry with Kim-wipes

Chain of Custody Form

REQUEST ID:
SAMPLING LOCATION:

Date/Time	SAMPLE IDENTIFICATION	MATRIX	NUMBER of CONTAINERS	PRESERVATION/ TREATMENT	ANALYSIS REQUESTED *A- Add D- Delete X- Select*	LAB USE ONLY
Date: __/__/__ Time: ___:___ MILITARY	ID#__ __ __ __ __ __ __ __ __	☐ WATER __Surface __Ground __Storm ☐ SOIL ☐ SEDIMENT ☐ OTHER	__ Glass, 1000 mL __ Plastic, 1000 mL __ Glass, 250 mL __ Plastic, 250 mL __ Plastic, 125 mL __ ISCO __ Glass, 60 mL __ Vial, 40 mL __ Vacutainer Other:	__ ICE __ H2SO4 __ HNO3 __ HCl __ H3PO4 __ RAW __ FILTERED __ GROUND	__ POC __ PN __ NO3 __ DP OTHER __ $\delta^{15}N\ Sed, \delta^{13}C\ Sed$ __ $\delta^{15}N_{NO_3^-}, \delta^{18}O_{NO_3^-}$ __ $\delta^{2}H_{H_2O}, \delta^{18}O_{H_2O}$ __ $\delta^{13}C_{DIC}$	LAB ID: COMMENTS:
Date: __/__/__ Time: ___:___ MILITARY	ID#__ __ __ __ __ __ __ __ __	☐ WATER __Surface __Ground __Storm ☐ SOIL ☐ SEDIMENT ☐ OTHER	__ Glass, 1000 mL __ Plastic, 1000 mL __ Glass, 250 mL __ Plastic, 250 mL __ Plastic, 125 mL __ ISCO __ Glass, 60 mL __ Vial, 40 mL __ Vacutainer Other:	__ ICE __ H2SO4 __ HNO3 __ HCl __ H3PO4 __ RAW __ FILTERED __ GROUND	__ NH4 __ NO3 __ DIC __ DOC __ DP __ δ15N NH4 __ δ15N NO3 __ δ15N Sediment __ δ13C Sediment __ POC __ PN OTHER	LAB ID: COMMENTS:
Date: __/__/__ Time: ___:___ MILITARY	ID#__ __ __ __ __ __ __ __ __	☐ WATER __Surface __Ground __Storm ☐ SOIL ☐ SEDIMENT ☐ OTHER	__ Glass, 1000 mL __ Plastic, 1000 mL __ Glass, 250 mL __ Plastic, 250 mL __ Plastic, 125 mL __ ISCO __ Glass, 60 mL __ Vial, 40 mL __ Vacutainer Other:	__ ICE __ H2SO4 __ HNO3 __ HCl __ H3PO4 __ RAW __ FILTERED __ GROUND	__ NH4 __ NO3 __ DIC __ DOC __ DP __ δ15N NH4 __ δ15N NO3 __ δ15N Sediment __ δ13C Sediment __ POC __ PN OTHER	LAB ID: COMMENTS:

Sampler's Signature:

Relinquished by:	Date:	Recieved by:	Date:
Representing:	Time:	Laboratory:	Time:

Archiving/Disposal (Notes/Dates):
Signature

Ion Chromatography of Water

1. Discussion

Principle

This method addresses the sequential determination of the following inorganic anions: *bromide, chloride, fluoride, nitrate, Kjeldahl nitrogen, total nitrogen* and *sulfate*. A small volume of water sample is injected into an ion chromatograph to flush and fill a constant volume sample loop. The sample is then injected into a stream of carbonate-bicarbonate eluent. The sample is pumped through three different ion exchange columns and into a conductivity detector. The first two columns, a precolumn (or guard column), and a separator column, are packed with low-capacity, strongly basic anion exchanger. Ions are separated into discrete bands based on their affinity for the exchange sites of the resin. The last column is a suppressor column that reduces the background conductivity of the eluent to a low or negligible level and converts the anions in the sample to their corresponding acids. The separated anions in their acid form are measured using an electrical conductivity cell. Anions are identified based on their retention times compared to known standards. Quantitation is accomplished by measuring the peak area and comparing it to a calibration curve generated from known standards.

Sensitivity

Ion Chromatography values for anions ranging from 0 to approximately 40 mg/L can be measured and greater concentrations of anions can be determined with the appropriate dilution of sample with deionized water to place the sample concentration within the working range of the calibration curve.

Interferences

Any species with retention time similar to that of the desired ion will interfere. Large quantities of ions eluting close to the ion of interest will also result in interference. Separation can be improved by adjusting the eluent concentration and /or flow rate. Sample dilution and/or the use of the method of Standard Additions can also be used. For example, high levels of organic acids may be present in industrial wastes, which may interfere with inorganic anion analysis. Two common species, formate and acetate, elute between fluoride and chloride. The water dip, or negative peak, that elutes near, and can interfere with, the fluoride peak can usually be eliminated by the addition of the equivalent of 1 mL of concentrated eluent (100X) to 100 mL of each standard and sample. Alternatively, 0.05 mL of 100X eluent can be added to 5 mL of each standard and sample.

Because bromide and nitrate elute very close together, they can potentially interfere with each other. It is advisable not to have Br-/NO3- ratios higher than 1:10 or 10:1 if both anions are to be quantified. If nitrate is observed to be an interference with bromide, use of an alternate detector (e.g., electrochemical detector) is recommended.

Method Interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms. Samples that contain particles larger than 0.45 micrometers and reagent solutions that contain particles larger than 0.20 micrometers require filtration to prevent damage to instrument columns and flow systems. If a packed bed suppressor column is used, it will be slowly consumed during analysis and, therefore, will need to be regenerated. Use of either an anion fiber suppressor or an anion micro-membrane suppressor eliminates the time-consuming regeneration step by using a continuous flow of regenerant.

Because of the possibility of contamination, do not allow the nitrogen cylinder to run until it is empty. Once the regulator gauge reads 100 kPa, switch the cylinder out for a full one. The old cylinder should them be returned to room #19 for storage until the gas company can pick it up. Make sure that the status tag marks the cylinder as "EMPTY".

Sample Handling and Preservation

Samples should be collected in glass or plastic bottles that have been thoroughly cleaned and rinsed with reagent water. The volume collected should be sufficient to ensure a representative sample and allow for replicate analysis, if required. Most analytes have a 28 day holding time, with no preservative and cooled to 4°C. Nitrite, nitrate, and orthophosphate have a holding time of 48 hours. Combined nitrate/nitrite samples preserved with H_2SO_4 to a pH $\leq$2 can be held for 28 days; however, pH$\leq$2 and pH$\geq$12 can be harmful to the columns. It is recommended that the pH be adjusted to pH$\geq$2 and pH$\leq$12 just prior to analysis.

Note: Prior to analysis, the refrigerated samples should be allowed to equilibrate

to room temperature for a stable analysis.

2. Apparatus

Dionex DX500

Dionex CD20 Conductivity Detector

Dionex GP50 Gradient Pump

Dionex Eluent Organizer

Dionex AS40 Automated Sampler

Dionex ASRS-Ultra Self-Regenerating Suppressor

Dionex Ionpac Guard Column (AG4A, AG9A, or AG14A)

Dionex Ionpac Analytical Column (AS4A, AS9A, or AS14A)

Dionex Chromeleon 6.8 Software Package

Dionex 5 mL Sample Polyvials and Filter Caps

2 L Regenerant Bottles

5 mL Adjustable Pipettor and Pipettor Tips

1 mL Adjustable Pipettor and Pipettor Tips

A Supply of Volumetric Flasks ranging in size from 25 mL to 2 L

A Supply of 45 micrometer pore size Cellulose Acetate Filtration Membranes

A Supply of 25x150 mm Test Tubes

Test Tube Racks for the above 25x150 mm Test Tubes

Gelman 47 mm Magnetic Vacuum Filter Funnel, 500 mL Vacuum Flask, and a Vacuum Supply

3. Reagents

Purity of Reagents—HPLC grade chemicals (where available) shall be used in all reagents for Ion Chromatography, due to the vulnerability of the resin in the columns to organic and trace metal contamination of active sites. The use of lesser purity chemicals will degrade the columns.

Purity of Water—Unless otherwise indicated, references to water shall be understood to mean Type I reagent grade water (Milli Q Water System) conforming to the requirements in ASTM Specification D1193.

Eluent Preparation for **SYSTEM2 NITRATE** *Methods, including Bromides (using AG4, AG4 and AS4 columns)*—All chemicals are predried at 105° C for 2 hrs then stored in the desiccator. Weigh out 0.191 g of sodium carbonate (Na_2CO_3) and 0.286 g of sodium bicarbonate ($NaHCO_3$) and dissolve in water. System 2 (the chromatography module that contains the AG4, AG4, and AS4 Dionex columns) to be sparged, using helium, of all dissolved gases before operation.

Eluent Preparation for **SYSTEM2 NITRATE** (F) *Method (using AG14 and AS14 columns)*— Weigh out 0.3696 g of sodium carbonate (Na_2CO_3) and 0.080 g of sodium bicarbonate (NaHCO3) and dissolve in water. Bring the volume to 1000 mL and place the eluent in the System 1 bottle marked for this eluent concentration. The eluent must be sparged using helium as in the above reagent for System 2.

Eluent Preparation for **SYSTEM2 TKN** (TKN) *Methods, including Total Nitrogen (using AG4A, AG4A, and AS4A columns)*—Weigh out 0.191 g of sodium carbonate (Na_2CO_3) and 0.143 g of sodium bicarbonate ($NaHCO_3$) and dissolve in water. Bring the volume up to 1000 ml and place in the System 2 bottle labeled "*IC-TKN 0.191/0.143*". Sparge the eluent as in the above reagent for System 2.

100X Sample Spiking Eluent—prepared by using the above carbonate/bicarbonate ratios, but increasing the concentration 100X. Weigh out 1.91 g of Na_2CO_3 and 2.86 g of $NaHCO_3$ into a 100 mL volumetric flask. 0.05 mL of this solution is added to 5 mL of all samples and standards to resolve the water dip associated with the fluoride peak.

Stock standard solutions, 1000 mg/L (1 mg/mL): Stock standard solutions may be purchased (SPEX) as certified solutions or prepared from ACS reagent grade materials (dried at 105° C for 30 minutes

Calibration Standards—for the **SYSTEM2 NITRATE** (except Bromide) methods are prepared as follows:

1. *Calibration Standard 1:* Pipette 0.1 mL of 1000 mg/L $NaNO_3$ stock standard, 0.1 mL of 1000 mg/L NaF stock standard, 2 mL of 1000 mg/L NaCl stock standard, and 10 mL of 1000 mg/L K_2SO_4 stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

2. *Calibration Standard 2*: Pipette 0.5 mL of 1000 mg/L $NaNO_3$ stock standard, 0.5 mL of 1000 mg/L NaF stock standard, 5 ml of 1000 mg/L NaCl stock standard, and 20 mL of 1000 mg/L K_2SO_4 stock standard into a 1000 mL volumetric flask, partially filled with water, then fill to volume.

3. *Calibration Standard 3*: Pipette 2.5 mL of 1000 mg/mL $NaNO_3$ stock standard, 2.5 mL of 1000 mg/L NaF stock standard, 10 mL of 1000 mg/L NaCl stock standard, and 40 mL of 1000 mg/L K_2SO_4 stock standard into a 1000 mL volumetric flask partially filled with deionized water, then fill to volume.

4. *Quality Control Sample:* Pipette 1.0 mL of 1000 mg/L $NaNO_3$ stock solution, 1.0 mL of 1000 mg/L NaF stock solution, 8 mL of 1000 mg/L NaCl stock solution, and 30 mL of mg/L K_2SO_4 stock standard into a 1000 mL volumetric flask, partially filled with water, then fill to volume.

Calibration Standards—for the **SYSTEM2 NITRATE** (Fluoride) method are prepared as follows:

1. *Calibration Standard 1*: Pipette 0.01 mL of 1000 mg/L NaF stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

2. *Calibration Standard 2*: Pipette 0.05 mL of 1000 mg/L NaF stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

3. *Calibration Standard 3*: Pipette 0.1 mL of 1000 mg/mL NaF stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

4. *Calibration Standard 4*: Pipette 0.5 mL of 1000 µg/mL NaF stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

5. *Calibration Standard 5*: Pipette 1.0 mL of 1000 mg/L 1000 stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

6. *Quality Control Standard*: Pipette 0.1 mL of 1000 mg/L NaF from a separate source stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

7. *Quality Control Standard*: Pipette 0.4 mL of 1000 mg/L NaF from a separate source stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

8. *Quality Control Standard*: Pipette 1.0 mL of 1000 mg/L NaF from a separate source stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volum

Calibration Standards—for the **SYSTEM2 NITRATE** (Bromide) method are prepared as follows:

1. *Calibration Standard 1*: Pipette 2 mL of 1000 mg/L NaBr stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

2. *Calibration Standard 2*: Pipette 5 mL of 1000 mg/L NaBr stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

3. <u>*Calibration Standard 3*</u>: Pipette 10 mL of 1000 mg/L NaBr stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

4. <u>*Quality Control Standard*</u>: Pipette 8 mL of 1000 mg/L NaBr stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

Outside Source Certified Quality Control Sample—ERA

4. Procedure

A. <u>Instrument Preparation</u>

1. Before turning on the Dionex Ion Chromatography System:
   a. Fill the eluent reservoir(s) with fresh eluent.
   b. Make certain the waste reservoir is empty of all waste.
   c. Turn on the helium. The system pressure should be between 7 - 15psi. The system pressure can be regulated with the knob on the back of the Eluent Organizer.
   d. Connecting a piece of tubing to the gas line going into the eluent bottle and putting the tubing into the eluent degasses the eluent reservoir(s). The gas knob on the Eluent Organizer that corresponds to the eluent bottle should be slowly opened until a constant bubbling stream can be seen in the eluent bottle.
   e. The eluent should be degassed with helium, for a minimum of 30 minutes, before operation of the instrument.
   f. After the eluent has been degassed, remove the tube from the eluent and tightly seal the eluent bottle. The eluent is now ready to introduce into the system.
2. Whether using the IP25 for Fluorides or the GP50 for everything else, turn off the browser, scroll to **REMOTE** on the screen, select **LOCAL** and **ENTER**.
3. Scroll to mL/min., change to 0 mL/min., and hit **ENTER**. If using the IP25 pump, skip to step #5.
4. Hit **MENU** and select **1**, then **ENTER**.
5. Insert syringe into the Priming Block, open the gas valve on the Eluent Organizer, turn the valve on the Priming Block counterclockwise, and turn on the pump that corresponds with the method to be ran by pushing the **OFF/ON** button.
6. If the syringe does not fill freely, assist by gently pulling back on the plunger of the syringe. Make certain that all of the air bubbles are removed from the eluent line to the pumps.
7. Press **OFF/ON** on the pump to turn it off.
8. Turn the valve on the Priming Block clockwise, remove the syringe and expel the air bubbles from the syringe.
9. Reinsert the syringe filled with eluent into the Priming Block.
10. Open the valve on the Pressure Transducer and the valve on the Priming Block with the eluent filled syringe still attached. This is accomplished by turning both counterclockwise.

11. Press **PRIME** on the pump and push the contents of the syringe into the Priming Block. After the eluent has been injected into the Priming Block, press **OFF/ON** to turn the prime pump off and to close the valves on the Pressure Transducer and Priming Block.
12. Remove the syringe from the Priming Block.
13. Scroll to the mL/min. on the screen for the pump. For the GP50, type 2 mL/min., and press **ENTER**. For the IP25, type 1.2 mL/min., and press **ENTER**.
14. Press **OFF/ON** to turn on the pump at the appropriate rate. The pressure should soon stabilize between both pumpheads after two minutes of pumping time.
15. If the pressure between pumpheads has a difference >20 psi, then shut down the pump and repeat steps 2-14 to remove air bubbles and prime the pumps.
16. Once the pump has a pumping pressure difference between pumpheads of <20 psi, then go to the computer and enter PeakNet.
17. On the computer**, turn on the Chromeleon 6.8 browser**, then choose either **System 1** (Fluoride) or **System 2** (all other anions including Bromide and TKN).
18. Go to **last run sequence, click to highlight and go to file, click save as.**. This will load the method of interest and a template for the current sequence run.
19. The sequence is edited to reflect the method and samples that are to be run.
    a. **SYSTEM2 NITRATE** for Fluoride
    b. **SYSTEM2 NITRATE** for Bromides
    c. **SYSTEM2 TKN** for TKN and Total Nitrogen

Note: Data is reprocessed in the section of Chromelon 6.8 called *Sequence integration editor*. Only operators with a <u>minimum of three months</u> experience in Ion Chromatography should attempt to reprocess data for this analysis. Once data is optimized, then the nitrogen values from nitrate and nitrite analysis can be subtracted from this value for the TKN nitrogen value. If only Total Nitrogen is needed then use the optimized data value without the correction for nitrite and nitrate nitrogen.

    d. **SYSTEM 2 NITRATE** for all other anions,

20. Observe the reading on the screen of the CD20 Conductivity Detector. A conductivity rate change of <0.03 μS over a 30 second time span is considered stable for analysis.
21. If using the GP50 pump, it will take about 15-30 minutes for the CD20 system to stabilize. If using the IP25, it will take between 30 minutes to 2 hours for stabilization.
22. Once the CD20 is stabilized, the Dionex DX500 Ion Chromatography

System is ready to start standardization.

NOTE: When using the GP50 Gradient Pump, all due care must be taken before one switches from local procedures to remote procedures. The bottle from which the eluent is being pumped (i.e., A, B, C, or D) must exactly match the bottle specified in the method. If there is a difference, then once the

pump control is turned over to remote control, irreversible damage and destruction of suppressors, columns, piston seals, and check valves on the GP50 Gradient Pump will occur. NEVER switch from bottle C to A, B, or D without flushing the system lines with water to remove all traces of eluent from bottle C from the lines.

B. Sample Preparation

1. If the sample was not filtered in the field, it must be done so now. Transfer 50 mL of a well-mixed sample to the filtering apparatus. Apply the suction and collect the filtrate.
2. If the conductivity values for the sample are high, dilution will be necessary to properly run the sample within the calibration standard range. Dilutions are made in the Polyvials with the plastic Filter Caps. If the dilutions are > 20X, then volumetric glassware is required.
3. All dilutions are performed with reagent grade DI water. Be sure to mix the dilution well.
4. For Fluorides and Bromides, pipette 5.0 mL of the filtered samples into the Polyvials. For all other anions, including TKN and Total Nitrogen, first pipette 0.05 mL of 100X sample spiking eluent into the Polyvials, then pipette 4.95 mL of the filtered samples on top of the spiking eluent.
5. The Filter Caps are pressed into the Polyvials using the insertion tool.
6. Place the Polyvials into the Sample Cassette, which is placed into the Autosampler.
7. The white/black dot on the Sample Cassette should be located on right-hand side when loaded in the left-hand side of the Automated Sampler for System 2.
8. For every ten samples the following should be included:
   a. 1 DI water blank
   b. 1 Duplicate of any one sample
   c. 1 Quality Control sample/calibration check

C. Calibration and Sample Analysis

1. Set up the instrument with proper operating parameters established in the operation condition procedure
2. The instrument must be allowed to become thermally stable before proceeding. This usually takes 1 hour from the point on initial degassing to the stabilization of the baseline conductivity.
3. To run samples on the Dionex Ion Chromatography System:
   a. Make a run schedule on the Chromeleon 6.8 Software Section labeled **SEQUENCE**.
   b. Double click the mouse on the **SYSTEM 1 SEQUENCES or SYSYTEM 2 SEQUENCES** to display the Scheduler Area. The name of the calibration standards must be entered under the sample name section as **Standard #1, Standard #2**, and **Standard #3**.

Note: Level must be changed to the corresponding standard level or the calibration

will be in error. (Example: Standard #1 = Level #1; Standard #5 = Level #5)

c. Next, enter QC, blanks, QC, samples, duplicates, QC, and blanks, in that order.

d. Under sample type, click on either **Calibration Standard** or **Sample**, depending on what is being run.

e. Under the **Method** section, the method name must be entered. To do so, double click on the highlighted area under **Method**, scroll through the list of methods and double click on the method of interest.

f. Next under the **Data File** section, enter the name of the data file.

g. Finally, in the **Dil** area, type in the dilution factor if different from 1. Do this for all standards, blanks, quality controls, duplicates, and samples to be run under this schedule.

h. Save the schedule and obtain a printout of it.

i. Standardize the Dionex Ion Chromatography System by running the standards: **Standard #1**, **Standard #2**, and **Standard #3**.

4. Run the QC standards.

5. Run the prepblank and DI water blank.

6. Run the samples, duplicates, and blanks.

7. Run the QC standards at the end.

5. Calculations

A. Calculations are based upon the ratio of the peak area and concentration of standards to the peak area for the unknown. Peaks at the same or approximately the same retention times are compared. Once the method has been updated with the current calibration, this is calculated automatically by the software using linear regression. Remember that when dilutions are being run, the correct dilution factor must be entered.

B. Manual calculations are based upon the ratio of the peak and concentration of standards to the peak area for the unknown when the software will not automatically calculate the unknown concentration. Peaks at the same or approximately the same retention times are compared. The unknown concentration can be calculated from using this ratio. Remember that when dilutions are being run that the correct dilution factor must be entered before you will get the correct result.

C. When possible the unknown should be bracketed between two knowns and the calculation of the unknown made from both for comparison.

6. Quality Control

A quality control sample obtained from an outside source must first be used for the initial verification of the calibration standards. A fresh portion of this sample should be analyzed every week to monitor stability. If the results are not within +/- 10 % of the true value listed for the control sample, prepare a new calibration standard and recalibrate the instrument. If this does

not correct the problem, prepare a new standard and repeat the calibration. A quality control sample should be run at the beginning and end of each sample delivery group (SDG) or at the frequency of one per every ten samples. The QC's value should fall between ± 10 % of its theoretical concentration.

A duplicate should be run for each SDG or at the frequency of one per every twenty samples, whichever is greater. The RPD (Relative Percent Difference) should be less than 10%. If this difference is exceeded, the duplicate must be reanalyzed.

From each pair of duplicate analytes (X_1 and X_2), calculate their RPD value:

$$\% \ RPD = 2 \bullet \left(\frac{X_1 - X_2}{X_1 + X_2} \right) x \, 100$$

where:

(X_1 - X_2) means the absolute difference between X_1 and X_2.

7. Method Performance

The method detection limit (MDL) should be established by determining seven replicates that are 2 to 5 times the instrument detection limit. The MDL is defined as the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

$$MDL = t_{(n-1,1-\alpha \ =99)}(S)$$

where:

t = the t statistic for n number of replicates used (for n=7, t=3.143)

n = number of replicates

S = standard deviation of replicates

8. Reference

EPA SW 846-9056, Chapter 5, September 1994

U.S. EPA Method 300.0, March 1984

ASTM vol. 11.01 (1996), D 4327, "Standard Test Method for Anions in Water by Chemically

Suppressed Ion Chromatography". 0/2010 addendum to 01/2009 Ion Chromatography of Water

1. Discussion

Principle and iodine.

3. Reagents

Calibration Standards

1. Calibration Standard 1: Pipette 0.1 mL of 1000 mg/L I stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
2. Calibration Standard 2: Pipette 0.5 mL of 1000 mg/L I stock standard into a

1000 mL volumetric flask partially filled with water, then fill to volume.

3. Calibration Standard 3: Pipette 1.0 mL of 1000 mg/L I stock standard into a

1000 mL volumetric flask partially filled with water, then fill to volume.

4. Calibration Standard 4: Pipette 5.0 mL of 1000 mg/L I stock standard into a

1000 mL volumetric flask partially filled with water, then fill to volume.

5. Calibration Standard 5: Pipette 10.0 mL of 1000 mg/L I stock standard into a

1000 mL volumetric flask partially filled with water, then fill to volume.

6. Quality Control Sample: Pipette 5.0 mL of 1000 mg/L I stock standard into a

1000 mL volumetric flask partially filled with water, then fill to volume.

Total Organic Carbon in Water (TOC)/

Dissolved Organic Carbon in Water (DOC)

MDL= 0.30 mg/L

1. Discussion

Principle

The organic carbon in water and wastewater is composed of a variety of organic compounds in various oxidation states. Biological or chemical processes can oxidize some of these carbon compounds further. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) tests may be used to characterize these factions; however, the presence of organic carbon that does not respond to either the BOD or COD tests make them unsuitable for the measurement of total organic carbon. While, total organic carbon (TOC) is a more convenient and direct expression of total organic content than either BOD or COD, it does not provide the same kind of information. If a repeatable empirical relationship is established between either BOD or COD, and TOC, then the TOC can be used to estimate the accompanying BOD or COD. However, this relationship must be established independently for each set of matrix conditions, such as various points in a treatment process. Unlike BOD and COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements (i.e., nitrogen, hydrogen), or inorganics that can contribute to the oxygen demand measured by BOD and COD. TOC measurement does not replace BOD and COD testing.

Measurement of TOC is of vital importance to the operation of water treatment and waste treatment plants. Drinking water TOCs range from <100ug/L to > 25,00ug/L. Wastewater may contain very high levels of organic compounds TOC>100mg/L. The presence of these organic contaminants may serve as nutrient source for undesired biological growth and for drinking water they may react with disinfectants to produce potentially toxic and carcinogenic compounds.

To determine the quantity of organically bound carbon, the organic molecules must be broken down and converted to a since molecular form. TOC methods convert organic carbon to carbon dioxide (CO2). It is more appropriate to use the High temperature combustion with Samples that have high levels of TOCs and or have complex matrix.
DOC is the same process just analyzed on a filtered sample. The sample should be filtered in the field with a GF/F filter pore size in the range of 0.7-0.25um. Sample should also be preserved after filtering with H3PO4 as with the TOC sample.

Interferences

Removal of carbonate and bicarbonate by acidification and purging with purified gas results in the loss of volatile organic substances. The volatiles also can be lost during sample blending, particularly if the temperature is allowed to rise. Another loss can occur if carbon containing particulates are unable to enter the needle. Filtration, although sometimes necessary, when DOC is to be determined, can result in loss or gain of DOC.
The major limitation to high-temperature techniques is the magnitude and variability of the blank. With any organic carbon measurement, contamination during sample handling and treatment is a likely source of interference. This is especially true of trace analysis. Take extreme care in sampling, handling, and analyzing samples below 1 mg TOC / L.

Sample Handling and Preparation

DOC samples shall be filtered in the field with a GF/F filter with a pore size range of 0.7-0.25 um then acidified the same as the TOC sample below**.**

Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. All samples should be stored at 4° C with no headspace in the bottles, as this will reduce the chance of losing purgeable organics. If analysis cannot be performed within two hours of collection, the sample should be acidified to a pH of $\leq$ 2 with H_3PO_4. However, this acidification invalidates any inorganic carbon determination of the sample. TOC samples have a 28 day hold time.

2. Safety

Phosphoric acid (H_3PO_4) is used in this method. Utilize the proper safety equipment and procedures while performing this analysis.

3. Apparatus

Total organic carbon analyzer—Teledyne Tekmar TORCH
Tank of Ultra High Purity grade Compressed Air with regulator
Volumetric Glassware
Analytical Balance—capable of weighing to the nearest 0.0001 g

4. Reagents (Get Water directly from the Purification System)

Purity of Reagents—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. Other grades may be used, provided it is first ascertained that the reagent is sufficiently high in purity to permit its use without lessening the accuracy of the determinations.

Purity of Water—Unless otherwise indicated, references to water shall be understood to mean Type 1 reagent grade water (Milli Q Water System) conforming to the requirements in ASTM Specification D1193.

Acid reagent-18 mL of 85% phosphoric acid (H_3PO_4)
94 ml of ultra pure water

TOC stock solution (1000 mg/L)—Dissolve 2.125 g of predried KHP in ultra pure water and dilute to a final volume of 1000 mL. Good for 1 month when stored between 2-8C

TOC standard solution (20 mg/L)—Dilute 5 mL of the TOC stock solution (1000 mg/L) to 250 mL with ultra pure water.

TOC standard solution (10 mg/L)— Dilute 2 mL of the TOC stock solution (1000 mg/L) to 200 mL with ultra pure water.

Quality Control Samples— Order from ERA Dilute to known concentration using instructions

5. Procedure

A. Perform Instrument checks -(Preventative Maintenance Chart in drawer)
Daily-
Weekly-
Monthly-
Date all tasks that were performed and initial

B. Determine your calibration range and pour chosen stock standard into bottle in position B. Normally this is a 20 ppm Stock. Instrument will dilute this stock to chosen calibration points.

C. **Set up New Calibration**

1. New
   Calibration
   TOC
   (Name Calibration ex. TOC today's date)
   OK
2. Open
   Method
   TOC Drinking Water -0.75mls
   Ok
   Select (at the top right of screen)
   Choose the name of calibration you just created
   Ok
   SAVE you must save or calibration will not work. **Use the Disk Save icon to save**

D. **Set up Schedule**
New
Schedule
Under sample Type choose
Clean – 2 reps
Clean – 2reps
Blank- click on Method area and choose TOC Drinking Water-0.75mls -3 reps
Blank- click on Method area and choose TOC Drinking Water- 0.75mls - 3reps
(*Instrument auto blank corrects*)
Cal Standard- choose "TOC 0.5-20.0 with the method that says TOC Drinking Water 0.75"
Select **Position should be B or wherever you placed your 20ppm stock**
3 reps per calibration point
Clean - 3 reps
Sample -Position of vial, ex.# 1&2 will be a known value QC 5 ppm and 10 ppm made up from other source than the stock used to make the calibration.
Sample –Position #3, name it, then choose Method (same as blank and calibration set) – 3reps.
After all samples are entered with appropriate positions, methods, and reps
Clean -3reps

** Using the last calibration ran.**- Can't be older than 2 months old.
Don't do a Cal Standard just run a known QC-for calibration check- after your blank, if it passes continue on with run if it fails stop run and recalibrate.

6. Calculations

Instrument auto blank corrects. This is why you only run a blank at the beginning of the run before the calibration and no more during the same run.

7. Quality Control

The quality control sample set should be run at the beginning and end of each sample group to be analyzed and at the frequency of one set per every ten samples. Each QC's value should fall between ± 10 % of its theoretical concentration.

The initial calibration verification QC sample should be run at the beginning of the day's analysis. The QC's value should fall between ± 10 % of its theoretical concentration.

A duplicate should be run at the end of each sample delivery group (SDG) or at the frequency of one per every ten samples, sufficient sample volume permitting. The RPD (Relative Percent Difference) should be less than 10%. If this difference is exceeded, the sample must be reanalyzed.

From each pair of duplicate analytes (X_1 and X_2), calculate their RPD value:

$$\% \ RPD = 2 \bullet \left(\frac{X_1 - X_2}{X_1 + X_2} \right) x\, 100$$

where:

(X_1 - X_2) means the absolute difference between X_1 and X_2.

8. Method Performance

The method detection limit (MDL) should be established by determining seven replicates that are 2 to 5 times the instrument detection limit. The MDL is defined as the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

$$MDL = t_{(n-1,1-\alpha=99)}(S)$$

where:

t = the t statistic for n number of replicates used

n = number of replicates

S = standard deviation of replicates

9. References

EPA SW 846-9060A, September 1986.

U.S. EPA 415.1, December 1982.

Standard Methods for the Examination of Water and Wastewater, 20th edition (1998), Method 5310-B, pg. 5-20-21.

01-09

Dissolved Inorganic Carbon SOP

1. Discussion

Principles

Dissolved Inorganic Carbon (DIC) is all inorganic carbon (e.g., carbon dioxide) dissolved in a given volume of water at a particular temperature and pressure.

Carbon dioxide gas evolved by dissolution in acid from carbonates In the sample is swept by a gas stream into a coulometer cell. The coulometer cell is filled with a partially aqueous medium containing ethanolamine and a colorimetric indicator. Carbon dioxide is quantitatively absorbed by the solution and reacts with the ethanolamine to form a strong, titratable acid which causes the indicator color to fade. The titration current automatically turns on and electrically generates base to return the solution to its original color (blue).

The coulometric determination of carbon dioxide has the unique distinction of performing with high degree of both precision and accuracy while maintaining relatively high sample throughput.

Working Range

<1 microgram up to 10,000 micrograms of Carbon for a single sample.

Interference

Coulometric system should remain a closed system. Outside air entering into the system after it has been purged will affect the results.

Sample Handle and Preparation

Sample should be taken to fill the bottle with no headspace, kept refrigerated at 4ºC and should not be opened until time of analysis. Sample should be analyzed ASAP from the time of collection

2. Safety

Safety glasses and gloves, and lab coat should be worn while performing this analysis due to the use of and possible exposure to strong acids and Silver Nitrate.

3. Apparatus

UIC Carbon Dioxide Coulometer CM5014

Becton Dickinson 5ml Syringes

4. Reagents

10% Phosphoric Acid – 50mls of O-Phosphoric Acid 85% in 450 mls of Mili-Q water

0.4M $AgNO_3$ Solution – 34g $AgNO_3$ in 500 mls of Milli-Q Water

Potassium Iodide (crystals) Fisher Brand – Bought from Fisher

UIC Carbon Anode Solution - Bought only from UIC

UIC Carbon Cathode Solution – Bought only from UIC

5. Procedure

A. Instrument Preparation

1. Check frit end is clean located in the back chamber in the $AgNO_3$ solution. ***If dirty then it must be cleaned, follow frit cleaning procedure Appendix A***
2. Check and fill titration bottle with 10% Phosphoric Solution
3. Remove or place a clean sample vial that will be used for acid blank reading.

B. Prepare Coulometer pH cup.

1. Wipe cup with kimwips to make sure there are **no fingerprints** or dust on cup. **(AVOID TOUCHING LARGE PART OF CUP)**
2. Large-cup – fill approximately 75mls with UIC Cathode Solution. Gently place the top on the cup, containing electrodes and air dispenser. Turn to have air dispenser toward the back of cup.
3. Arm of cup – Poor a layer of Potassium Iodide to approximately ¼ up the membrane between large cup and arm. Fill the arm with anode solution to equal level of solution in large cup. Gently place in silver electrode. **(DO NOT Touch Potassium Iodide)**
4. Place cup in the coulometer and attach the electrodes and the air fittings to their appropriately colored connections on the coulometer.

C. Starting the Coulometer

1. Turn on the water from the hood so that there is a constant drip running through coulometer and into the sink behind the instrument.
2. Turn on the gas 1.5 twists.
3. Turn on Titrator apparatus. Check flow meter it should be reading approximately 100.
4. Turn on power to coulometer. CELL BUTTON SHOULD STILL BE IN OFF POSITION.
5. Hit down arrow key ↓
6. Select Run Diagnostics
7. Select # 3 Set date and Time (set date and time used full year example 2008) and 00 for seconds
8. Select change Settings answer the questions as follows

- Carbon

- Weight

- Milligrams

-0.7

- 1.00

- 6

- 1.00 (minutes)

- Coulometer end point

- Manual

- N

9. Select Print Settings

10. Select Exist Diagnostics

11. Select Run Cell Set-up

- Move cell around until you the cell to read as close to 3950 without going over once there press F2

13. Turn Cell button to on

14. Select Run Analysis

15. Wait approximately 30 minutes until the %T reading is at 29

16. When reading is at 29% press enter to start run

D. Running Samples

1. Blank will **ALWAYS** be first. Blank is the empty vial with stir rod place on during instrument set-up. Sample ID will be "BLANK" and it will not give you opportunity to put in weight. It will go right to place to, pipette in the acid (6 mls) from titrator bottle and hit enter QUICKLY. **Blank should always read less then 7.**

2. QC is the standard $CaCO_3$ Sample ID $CaCO_3$ Press enter. Enter weight in mg press enter. Put in acid from titrator bottle and then press enter quickly. *%C should be between 11.7-12.1*

3. If you are running solid sample weight out and follow the same procedure as the QC/ Standard.

4. If you are doing DIC –water samples then follow rest of this procedure

5. Place a clean vial on with stir rod.

Enter sample ID press enter. Enter weight or volume ml=mg. Use 3 to 5mls of sample pulled from sample bottle into a syringe. Titrate 3mls of acid into vial, inject sample into top of cylinder press enter and titrate another 3mls in quickly. Let coulometer run until a result is reached. This result is in %C.

6. Run each water sample in this way with duplicates at least every 10 samples preferably every 5. Use a new vial for each new sample and or aliquot. **Between each sample it will ask if you want to run another sample. Always select yes until you are finished.**

7. After running the last sample / QC select no to more samples and the coulometer will print final results page.

F. Breaking down Coulometer

1. Turn off Titrator / flow unit
2. Turn off the coulometer unit
3. Turn off gas and water to unit
4. Remove the cup from unit
5. Empty the cup contents in the blue hazardous drum.
6. Wash cup (do not use anything that would scratch glass) and rinse VERY WELL with Milli-Q water and place on tray to dry. Rinse all other parts off with Milli-Q and place on tray to dry.

6. Calculations

The value from the Coulometer is in Micrograms C.

Conversion to ppm C (DIC) in solution

$$\frac{\text{Coulometer reading – blank reading (of acid and vial)} * 1 \text{ (density of water)}}{\text{Mls of sample injected into coulometer}}$$

Conversion to ppm CO_2 in solution

*ppm C (DIC) * 3.6658 = ppm CO_2 in solution*

7. Quality Control / Rate and Range

"This 100% efficient coulometric process gives results in basic theoretical units (coulombs) so calibration using standards is not required.

"The linear range and accuracy (better than 0.20% relative standard deviation for standard materials) of the coulometer generally exceeds that obtained by other detection methods."

"Working range of the CO_2 Coulometer is from less than one microgram C up to 10,000 micrograms of C for a single sample"

"Coulometer cell solution has an absorbance capacity of over 100mg for a single cell filling, typically allowing for a full day of sampling."

"Titrating at its max current (200ma) the CO_2 Coulometer can titrate approximately 1500 micrograms of carbon (5500ug of CO_2) per minute."

QC checks are measuring a standard of Calcium Carbonate.

Standard =12.0 %C

Acceptable Range = 11.7-12.1

Trouble Shooting- If qc's are not coming out

- Check to make sure there are not leaks in system (mainly at vial and screw-top lid.

- Check gas pressure and water pressure

- Another problem could be the weight. If samples are not weighed out properly, bad calibrated balance, sample results will not be accurate

- After checks run another qc sample if still not acceptable turn off instrument process will have to be started again from the beginning with new cell material

At *this point check the silver probe it may need replacing.*

8. Method Performance

MDL studies are not performed on this instrument based on the low range and the fact that it is not a calibrated instrument.

Repeatability of this instrument

Standard Deviation of at least 7 replicate readings of the QC ($CaCO_3$)

Task performed every 3 to 6 months.

9. References

UIC Carbon Dioxide Coulometer Application Note 1

UIC Carbon Dioxide Coulometer Application Note 3

Frit Cleaning Procedure

1. Remove the Frit and place in a small container of 9M HCL. Allow Frit to sit and with a bulb pull some of the HCL through the fit and empty into a HCL waste container. Should notice frit becoming lighter in color.

2. **Rinse the frit WELL** Pull clean Milli-Q water up through the fit and empty into waste container over and over. This process takes quite a few times.

3. Test the water from the fit on pH strips to make sure there is no residual acid present.

4. Empty the old AgNO3 solution into hazardous waste drum and fill approximately 1 inch of new AgNO3 solution.

5. Attach the frit apparatus back onto the coulometer.

Make sure you keep track of where the hoses belong when removing and reattaching the frit apparatus

Ammonia as Nitrogen in Water

1. Discussion

MDL = 0.02 as of 5/2002

Principle

An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside.

Sensitivity

This method covers the range from 0.05 ppm to 1.00 ppm ammonia as nitrogen.

Interferences

Complexing magnesium and calcium with citrate eliminates interference produced by precipitation of these ions at high pH. There is no interference from other trivalent forms of nitrogen.

Sample Preservation

Samples may be preserved up to 28 days by adding concentrated sulfuric acid to adjust to pH 2 or less and refrigerating at 4°C.

2. Safety

Phenol is volatile, corrosive, and toxic. Use with proper ventilation and protective gear.

3. Apparatus

Varion 50 Spectroscopy system
Magnetic stirrer
Filtration apparatus:
Gelman 47 mm magnetic filter funnel.
Suction flasks, connected in series to a vacuum system.
Reservoir for the filtrate, 500 mL.
Trap which prevents liquid from entering the vacuum system, 1000 mL.
Glass fiber filters—Whatman 47 mm, 1 μm glass fiber filters.

4. Reagents

Purity of Reagents—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. Other grades may be used, provided it is first ascertained that the reagent is sufficiently high in purity to permit its use without lessening the accuracy of the determinations.

Purity of Water—Unless otherwise indicated, references to water shall be understood to mean Type I reagent water conforming to the requirements in ASTM Specification D1193.

Sodium hydroxide solution, 1 N—Dissolve 40 g of NaOH in 500 mL of water. Dilute to 1 L.

Sulfuric acid solution, 1 N—Slowly add 28 mL of concentrated H_2SO_4 to 500 mL of water. Dilute to 1L.

Sodium hydroxide solution, 10 N—Dissolve 400 g of NaOH in 800 mL of water. Dilute to 1 L.

Sodium hypochlorite—5% solution that is available as commercial bleach. Purchase fresh bleach

every two months.

Alkaline citrate—Dissolve 100 g of trisodium citrate and 5 g of sodium hydroxide in water. Dilute to 500 mL.

Phenol solution—Mix 11.1 mL phenol (≥89%) in ethanol (95%) to a final volume of 100 mL. Store out of light in a tin canister. This reagent **must be prepared weekly**.

CAUTION: Phenol is volatile and toxic. Use with proper ventilation and protective gear.

Oxidizing solution—Mix one part of the bleach with four parts of the alkaline citrate solution. **Prepare fresh daily.**

Sodium nitroprusside solution—0.05% solution purchased from LabChem, Inc., or prepared by dissolving 0.5 g sodium nitroprusside in 1 liter of water. Store in a dark bottle for up to a month.

Stock ammonia as nitrogen solution—Purchased 1000 mg/L ammonia as nitrogen standard. (Fisher #13-641-924C).

Ammonia standard, 5 mg/L—Dilute 1 mL of the 1000 mg/L stock ammonia solution to 200 mL with water adjusted to a pH of 2 or less.

Blank—water adjusted to a pH of 2 or less. (This will have all reagents added in the same manner as the standards and samples.)

Ammonia QC Stock Solution—Using a commercially available quality control solution, dilute to a desired range and record manufacturers name, lot #, and date.

Quality control sample—Dilute ammonia QC stock solution so that QC value falls midway in analysis working range (0.05-1.00 ppm). Using 18 ppm QC stock solution, dilute 5 mL of ammonia stock to 250 mL, resulting in a concentration of 0.36 ppm.

5. Procedure

A. Standards Prep

1. Prepare standard concentrations, as described below, using the ammonia standard (5 mg/L) and diluting them to a volume of 50 mL with water of a pH ≤ 2. This is necessary if samples have been preserved with H_2SO_4..

Note: 50 drops of concentrated H_2SO_4 in 1 L of DI water yields the desired pH.

Volume of Ammonia standard, mL	Standard concentration, mg/L
0.5	0.05
1	0.10
3	0.30
5	0.50
8	0.80
10	1.00

2. Standards must be prepared **daily**.
3. The intense color development at concentrations greater than 0.8 ppm will be related in a curvilinear fashion. If it is necessary to work in ranges greater than 1.0ppm, it is

   important to remember this.

   Do not accept any result outside the last point on the calibration curve. Sample must be diluted (to measures inside the 0.5-1.0ppm curve) and ran again on a new run

B. Sample Prep
1. Pour 50 mL portions of all standards, samples, and QC's into 100 mL plastic beakers.
2. Add 1 mL of the EDTA solution, if deemed necessary.
3. Adjust all standards, samples, blanks, and QC's in the pH range 9-11with H_2SO_4 and or NaOH. The pH can be determined using the using multi-color plastic pH test strip.

Note: The color reaction is pH dependent, so this is CRITICAL.

4. Filter the standards, samples, and QC's.
5. Volumetrically transfer 25 mL of each adjusted sample, standard, blank, and QC's into a 25 mL beaker.
6. Place stir bars in each beaker.
7. Add the following reagents to each:
   a. 1 mL phenate solution
   b. 1 mL sodium nitroprusside solution
   c. 2.5 mL oxidizing solution
8. Cover with parafilm and place on stir plate. Develop for one hour at room temperature in subdued light. (Color is stable for 24 hrs.)

C. Sample Analysis
1. The spectrophotometer must be allowed to warm up for at least one hour before use. See Spectrophotometer SOP for a detailed listing of necessary computer commands.
2. For ammonia, the wavelength must be set to scan a range of **640nm.**.

Note: Phenol Waste from the this assay will react with the General Acidic Waste. KEEP THEM SEPARATE!!

3. Read and record absorbance on the spectrophotometer. This is usually done the morning following color development.
5. Pour leftover sample waste in phenate waste container.
6. For glassware clean up, refer to **"AMMONIA"** section of Glassware GLP.

D. Calculations
Results given are NH3-N (not NH3). Convert using NH3 = (NH3-N) / (0.8224**)**

6. Quality Control

A quality control sample should be run at the beginning and end of each sample delivery group (SDG) or at the frequency of one per every ten samples. The QC's value should fall between ± 10 % of its theoretical concentration.

A duplicate should be run for each SDG or at the frequency of one per every twenty samples, whichever is greater. The RPD (Relative Percent Difference) should be less than 10%. If this difference is exceeded, the duplicate must be reanalyzed

From each pair of duplicate analytes (X_1 and X_2) calculate their RPD value:

$$\% \ RPD = 2 \bullet \left(\frac{X_1 - X_2}{X_1 + X_2} \right) x\, 100$$

where:

(X_1 - X_2) means the absolute difference between X_1 and X_2.

If a sample's value exceeds 1.00 ppm, the sample must be diluted. The samples must be diluted so that its concentration falls between 0.05 ppm and 1.00 ppm. The sample must diluted using volumetric flasks and pipettes.

7. Method Performance

The method detection limit (MDL) should be established by determining seven replicates that are 2 to 5 times the instrument detection limit. The MDL is defined as the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

$$MDL = t_{(n-1,1-\alpha=99)}(S)$$

where:

t = the t statistic for n number of replicates used
n = number of replicates
S = standard deviation of replicates

8. References

Standard Methods for the Examination of Water and Wastewater, 20th edition (1998), Method 4500-NH_3-F, pg. 4-108

ASTM vol. 11.01 (1996), D 1193, " Specification for Reagent Water", pg. 116

More info on Ammonium and Ammonia is located on our local drive
G drive, Labworks, SOP, Waterpar, NH3NH4+info

Total Kjeldahl Nitrogen Preparation

1. Discussion

Principle
Total Kjeldahl Nitrogen is the sum of organic nitrogen and ammonia nitrogen compounds of a sample. This method oxidizes all of the organic and inorganic nitrogenous compounds, at 100 to 110ºC, to nitrate. The digestion also helps dissolve solid material that could interfere with obtaining an accurate reading. The total nitrogen is then determined by the analysis of nitrate in the digestate with an IC. Total Kjeldahl Nitrogen is then determined by subtracting the pre-determined nitrite plus nitrate nitrogen values from the total nitrogen values.

Sensitivity
This method covers the range from 0.1 ppm to 2.9 ppm.

Interferences
Since this method is designed to oxidize ammonia to nitrate for analysis, the use of ammonia and/or ammonia based substances should be avoided in the work area and on the glassware, as this could produce increased positive results that are inaccurate.

Sample Preservation
This method cannot be performed on samples preserved in acid. Because of this, the samples should be prepped ASAP.

2. Safety

Wear a lab coat, gloves, and protective eyewear when prepping this experiment to avoid possible exposure to harmful substances.

3. Apparatus

CEM MARS Microwave Digestion Unit
Advanced Composite Vessels (ACV)
Graduated Cylinder
Wash Bottle
Automatic Pipettor

4. Reagents

Purity of Reagents—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. Other grades may be used, provided it is first ascertained that the reagent is sufficiently high in purity to permit its use without lessening the accuracy of the determinations.

Purity of Water—Unless otherwise indicated, references to water shall be understood to mean Type I reagent grade water (Milli Q Water System) conforming to the requirements in ASTM Specification D1193.

Borate Buffer Solution—Dissolve 61.8 g H_3BO_3 and 8.0 g NaOH in a 1 L volumetric flask containing at least 500 mL of DI water. Swirl to mix and bring to volume. Make fresh

every 3 months.

Digestion Reagent—Dissolve 20.1 g of $K_2S_2O_8$ and 3 g of NaOH in a 1 L flask containing at least 500 mL of DI water. Swirl to mix and bring to volume. Make fresh every 3 months.

Quality Control—Commercially available wastewater TKN standard (Environmental Resource Associates, "Ready-To-Use Wastewater QC Standards", Cat # 743, Arvada CO, 1-800-ERA-0122)

Glutamic Acid Stock Standard ($C_3H_5NH_2$ (COOH) $_2$), 100 ppm—Dry Glutamic Acid in oven at 105°C for 24 hours. Cover and place in dissector until cool. Dissolve 1.051g in DI water and dilute to 1 L; preserve with 2 mL chloroform ($CHCl_3$). Store in refrigerator for no longer than 6 months.

Nitrate Stock Standard (NO_3-N), 1000 ppm—Dry Potassium Nitrate (KNO_3) in oven at 105°C for 24 hours. Cover and place in dissector until cool. Dissolve 0.7218g in DI water and bring to 1 L; preserve with 2 mL chloroform ($CHCl_3$). Store in refrigerator for no longer than 6 months.

Nitrate Working Standard, 10 ppm—Dilute 100 mL of Nitrate Stock Standard to 1000 mL in 1 L flask. Preserve with 2 mL chloroform (CHCl3). Store in refrigerator for no longer that 6 months.

5. Procedure

A. Turn on the CEM MSP 1000 Microwave Digestion Unit and allow it to warm up for at least 15 minutes.

B. Standards Prep
   1. *Using the 100 ppm Glutamic Acid Stock Standard, prepare the following*:
      a. 0.4 ppm = 1 mL of 100 ppm diluted to 250 mL
      b. 0.8 ppm = 2 mL of 100 ppm diluted to 250 mL
      c. 1.6 ppm = 4 mL of 100 ppm diluted to 250 mL
   2. *Using the 10 ppm Nitric Stock Standard, prepare the following*:
      a. 0.1 ppm = 1 mL of 10 ppm diluted to 100 mL
      b. 0.2 ppm = 2 mL of 10 ppm diluted to 100 mL
      c. 0.4 ppm = 4 mL of 10 ppm diluted to 100 mL
      d. 0.8 ppm = 8 mL of 10 ppm diluted to 100 mL
      e. 1.6 ppm = 16 mL of 10 ppm diluted to 100 mL
      f. 2.9 ppm = 29 mL of 10 ppm diluted to 100 mL
   3. *The QC is diluted from the ordered solution*: perform an appropriate dilution creating a QC with a value midway on calibration curve, (~1.5 ppm) using the ordered standard.
   4. If it is deemed necessary, ICV's (Initial Calibration Verification) and CCV's (Continued Calibration Verification) can be run using a 0.8 ppm and/or 1.6 ppm glutamic acid solution.

C. Sample Prep
   1. The Prep Blank is 10 mL of reagent grade DI water poured into the first liner.
   2. For all samples and QC, a 10 mL aliquot should be poured into one of the advanced composite vessels, or ACV, liners.
   3. Add 5 mL of Digestion Reagent to each liner.
   4. Assemble the ACV system as described in **Microwave Digestion GLP**.

D. Digestion Set Up
   1. From the options on the main menu of the microwave, press F3—"Recall Method/Data".

2. Press F1—"Recall Stored Method".
3. Use arrow keys to scroll down to "TKN SM"; press "Enter".
4. Press F1—"Load Program".
5. Press F4—"Start".
6. Press F1—"Yes". Once a digestion is started, watch the temperature probe and pressure tube carefully to make sure they do not become tangled up. If they do become tangled, press F1 to abort the run and remedy the problem.
7. Once the run is complete, disassemble the ACV's, add 1 mL of Borate Buffer Solution to each liner (all QC, samples, dups., etc.) and pour the digested samples into appropriately labeled precleaned containers.
8. The digested QC and samples, along with the corresponding data sheets, are to be transferred to the IC for analysis.

E. Prep-Batching

1. Log-on to the "Labworks" system.
2. Click on "Edit Data".
3. Enter the SDG number or choose it from the list.
4. Click on "OK".
5. Click on "OK".
6. In the row for TKN prep work (TKN_PREP), enter a 1 under the number of each sample completed and save it.
7. Exit system.

6. Quality Control

A duplicate sample should be prepped at the frequency of one per every twenty samples (sufficient sample permitting), or one per SDG, whichever is greater. The RPD should be less than 10%. If this difference is exceeded, the duplicate may need to be reprepped. The QC's value should fall between ± 10 % of its theoretical concentration as well.

7. References

Standard Methods for the Examination of Water and Wastewater, 20th edition (1998), Method 4500-N C, pg. 4-102

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2. Nominated for Graduate Student Representative for Civil Engineering Education Team (2018)
3. SENSE Fellowship Recipient, UK Civil Engineering NSF Fellowship (2017)
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Journal Publication

1. Mahoney, D. T., Al Aamery, N., Fox, J. F., Riddle, B., Ford, W., & Wang, Y. T. (2018). Equilibrium sediment exchange in the earth's critical zone: evidence from sediment fingerprinting with stable isotopes and watershed modeling. *Journal of Soils and Sediments*, 1-25.

Conference Presentations: Peer-Reviewed Abstract

1. Riddle, B., Monhollen, A., Fox, J.F., Wang, Y.T., Ford, W., Backus, J., Pollock, E. 2019. Insight to the mineralization of fine sediment organic matter in streams using stable isotope experiments, Kentucky Water Resources Annual Symposium, Lexington, KY, March 25, 2019.

2. Riddle, B., Fox, J.F., and Mahoney, D.T., Water supply impacted by algae and sedimentation in Kentucky: advancing sensors and nonconservative tracers, Kentucky Water Resources Annual Symposium, Lexington, KY, March 19, 2018.